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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 :
C12N 15/57, 9/64, 15/39
A61K 31/70, 37/02, C12Q 1/68
G01N 33/577, 33/68

A1

(11) International Publication Number: WO 93/25694

(43) International Publication Date: 23 December 1993 (23.12.93)

(21) International Application Number: PCT/US93/05705

(22) International Filing Date: 14 June 1993 (14.06.93)

(30) Priority data:
07/897,788 12 June 1992 (12.06.92) US
07/984,182 20 November 1992 (20.11.92) US

(71) Applicant: MASSACHUSETTS INSTITUTE OF TECHNOLOGY [US/US]; 77 Massachusetts Avenue, Cambridge, MA 02139 (US).

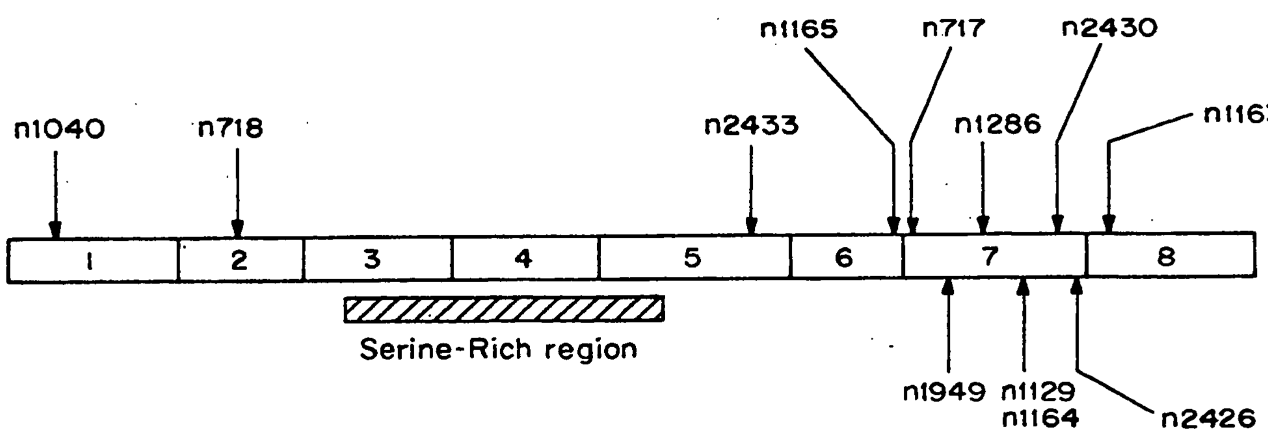
(72) Inventors: HORVITZ, H., Robert ; 80 Wndell Street, No. 2, Cambridge, MA 02138 (US). YUAN, Junying ; 22 Fairlee Road, Newton, MA 02168 (US). SHAHAM, Shai ; 132 Commonwealth Avenue, No. 9, Boston, MA 02116 (US).

(74) Agents: GRANAHAN, Patricia et al.; Hamilton, Brook, Smith & Reynolds, Two Militia Drive, Lexington, MA 02173 (US).

(81) Designated States: CA, JP, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: INHIBITORS OF CED-3 AND RELATED PROTEINS



(57) Abstract

Described herein is the discovery that human interleukin-1β convertase (ICE) is structurally similar to the protein encoded by the *C. elegans* cell death gene, *ced-3*. Comparative and mutational analyses of the two proteins, together with previous observations, suggest that the Ced-3 protein may be a cysteine protease like ICE and that ICE may be a human equivalent of the nematode cell death gene. Another mammalian protein, the murine NEDD-2 protein, was also found to be similar to Ced-3. The NEDD-2 gene is implicated in the development of the murine central nervous system. On the basis of these findings, novel drugs for enhancing or inhibiting the activity of ICE, *ced-3*, or related genes are provided. Such drugs may be useful for treating inflammatory diseases and/or diseases characterized by cell deaths, as well as cancers, autoimmune disorders, infections, and hair growth and hair loss. Furthermore, such drugs may be useful for controlling pests, parasites and genetically engineered organisms. Furthermore, novel inhibitors of the activity of *ced-3*, ICE and related genes are described which comprise portions of the genes or their encoded products.

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INHIBITORS OF CED-3 AND RELATED PROTEINS

Background

Cell death is a fundamental aspect of animal development. Many cells die during the normal development of both vertebrates (Glucksmann, *Biol. Rev. Cambridge Philos. Soc. 26*:59-86 (1951)) and invertebrates (Truman, *Ann. Rev. Neurosci.* 7:171-188 (1984)). These deaths appear to function in morphogenesis, metamorphosis and tissue homeostasis, as well as in the generation of neuronal specificity and sexual dimorphism (reviewed by Ellis *et al., Ann. Rev. Cell Biol.* 7:663-698 (1991)). An understanding of the mechanisms that cause cells to die and that specify which cells are to live and which cells are to die is essential for an understanding of animal development.

The nematode *Caenorhabditis elegans* is an appropriate organism for analyzing naturally-occurring or programmed cell death (Horvitz *et al., Neurosci. Comment. 1:56-65 (1982)*). The generation of the 959 somatic cells of the adult *C. elegans* hermaphrodite is accompanied by the generation and subsequent deaths of an additional 131 cells (Sulston and Horvitz, *Dev. Biol. 82*:110-156 (1977); Sulston *et al., Dev. Biol. 100*:64-119 (1982)). The morphology of cells undergoing programmed cell death in *C. elegans* has been described at both the light and electron microscopic levels (Sulston and Horvitz, *Dev. Biol. 82*:100-156 (1977); Robertson and Thomson, *J. Embryol. Exp. Morph. 67*:89-100 (1982)).

Many genes that affect *C. elegans* programmed cell death have been identified (reviewed by Ellis *et al., Ann. Rev. Cell Biol.* 7:663-698 (1991)). The activities of two of these genes, *ced-3* and *ced-4*, are required for the onset of almost all *C. elegans* programmed cell deaths (Ellis and Horvitz, *Cell 44*:817-829 (1986)).

When the activity of either *ced-3* or *ced-4* is eliminated, cells that would normally die instead survive and can differentiate into recognizable cell types and even function (Ellis and Horvitz, *Cell 44*:817-829 (1986); Avery and Horvitz, *Cell 51*:1071-1078 (1987); White *et al., Phil. Trans. R. Soc. Lond. B. 331*:263-271 (1991)). Genetic mosaic analyses have indicated that the *ced-3* and *ced-4* genes most likely act in a cell autonomous manner within dying cells, suggesting that the products of these genes are expressed within dying cells and either are cytotoxic molecules or control the activities of cytotoxic molecules (Yuan and Horvitz, *Dev. Biol. 138*:33-41 (1990)).

Summary of the Invention

This invention is based mainly on two experimental findings and their implications: 1) that human interleukin-1β convertase (ICE), which converts pro-interleukin-1β to the active cytokine and is involved in the inflammatory response in humans, has considerable similarity to the protein encoded by the *C. elegans* cell death gene, *ced-3*; and 2) that fusion constructs containing amino-terminal portions of the *ced-3* gene can prevent cell death in *C. elegans*. As discovered by Applicant, the human ICE and nematode Ced-3 proteins have an overall amino acid identity of 28%. A higher degree of similarity was found in the carboxyl-terminal region, a region shown to be critical for the activities of both proteins. Furthermore, three sequences important for ICE activity, the region surrounding the active cysteine and two autocleavage sites, have been shown to be conserved in the *ced-3* gene product.

Thus, significant structural similarity has been shown between two proteins which previously were thought to be unrelated (to have dissimilar physiological

roles). This finding leads to several implications, some of which are:

1) that the human ICE gene has an activity similar to that of *ced-3* in causing cell death;

2) that the Ced-3 protein is also a cysteine protease with a substrate specificity similar to that of ICE;

3) that mutations in the ICE gene corresponding to mutations in the *ced-3* gene will produce similar effects, such as inactivation and constitutive activation;

4) that the *ced-3* and ICE genes are members of a family of structurally related genes, referred to herein as the *ced-3*/ICE family, some of which are likely to be cell death genes and some of which may encode substrate-specific proteases;

5) that inhibitors of ICE, such as peptide aldehydes which contain the ICE recognition site or a substituted recognition site and the cowpox virus CrmA protein, may also be useful for inhibiting cell deaths; and

6) that inhibitors of *ced-3*, such as inhibitory portions of the gene or encoded product, may also be useful for inhibiting inflammation.

This hitherto unknown connection between a cell death protein and a protease involved in the inflammatory response provides a basis for developing novel drugs and methods for the treatment of acute and chronic inflammatory disease, of leukemias in which IL-1β is implicated, and of diseases and conditions characterized by cell deaths (such as myocardial infarction, stroke, traumatic brain injury, viral and other types of pathogenic infection, neural and muscular degenerative diseases, aging, hair loss). In addition, drugs which increase cell deaths and which are useful

for reducing the size or proliferative capacity of cell populations, such as cancerous cells, infected cells, cells which produce autoreactive antibodies, and hair follicle cells, as well as drugs which incapacitate or kill organisms, such as pests, parasites and recombinant organisms, can be developed using the *ced-3*, ICE, and other *ced-3*/ICE genes and their gene products.

This work also provides probes and methods for identifying additional members of the *ced-3*/ICE gene family. Genes related to *ced-3* and ICE are expected to exist in various organisms. Some of these may be cell death genes and/or proteases. The sequences of these related genes and their encoded products can be compared, for instance, using computer-based analysis, to determine their similarities. Structural comparisons, for example, would indicate those regions or features of the genes or encoded products which are likely to be functionally similar and important. Such information can be used to design drugs which mimic or alter the activity of the *ced-3*, ICE, or other *ced-3*/ICE genes, and which may, thus, be useful in the various medical and agricultural applications mentioned above.

In addition, another mammalian protein, the murine NEDD-2 protein, was also found to be similar to Ced-3. Interestingly, NEDD-2 is not significantly similar to ICE. Thus, another potential mammalian cell death gene was identified.

Also described herein is the discovery that fusion constructs which encode an amino-terminal portion of the Ced-3 protein fused to β-galactosidase act as inhibitors of cell death in *C. elegans*. Due to its structural similarity to Ced-3, constructs encoding corresponding portions of the human ICE protein are also expected to inhibit the enzymatic activity of ICE in cleaving interleukin-1β. Thus, inhibitors comprising an amino-

terminal portion of the Ced-3 protein, ICE protein or another member of the Ced-3/ICE family and RNAs and DNA constructs which express these protein portions are potentially useful for decreasing cell deaths and/or inflammation involved in various pathologies. Methods for identifying other inhibitory portions of the *ced-3* and ICE genes are also described.

Furthermore, deletion of the inhibitory amino-terminal portions of the *ced-3* and ICE genes may result in constitutive activation of the genes. Constitutively activated carboxyl-terminal portions of the genes, or their encoded products, may thus be useful in applications where increased cell deaths or an increased inflammatory response are desired.

Brief Description of the Drawings

Figure 1 shows the physical and genetic maps of the *ced-3* region on chromosome IV.

Figure 2 summarizes the experiments to localize *ced-3* within C48D1. Restriction sites of plasmid C48D1 and subclone plasmids are shown. Ced-3 activity was scored as the number of cell corpses in the head of L1 young animals. ++, the number of cell corpses above 10. +, the number of cell corpses below 10 but above 2. -, the number of cell corpses below 2.

Figures 3A-H show the nucleotide sequence (SEQ ID NO:1) of *ced-3* and deduced amino acid sequence (SEQ ID NO:2). The genomic sequence of the *ced-3* region was obtained from plasmid pJ107. The introns and the positions of 12 *ced-3* mutations are indicated. The likely translation initiation site is indicated by a solid arrowhead. The SL1 splice acceptor of the RNA is boxed. Repetitive elements are indicated as arrows above the relevant sequences. Numbers on the sides

indicate nucleotide positions. Numbers under the amino acid sequence indicate codon positions.

Figure 4A shows the genomic structure of the *ced-3* gene and the location of the mutations. The sizes of the introns and exons are given in bp. The downward arrows indicate the positions of 12 EMS-induced mutations of *ced-3*. The arrow pointing right indicates the direction of transcription. The solid arrowhead indicates the translation initiation site. The open arrowhead indicates the termination codon.

Figure 4B shows the locations of the mutations relative to the exons (numbered 1-7) and the encoded serine-rich region in *ced-3*.

Figure 5 is a Kyte-Doolittle hydrophobicity plot of the Ced-3 protein.

Figures 6A-B show the alignment of the amino acid sequences of Ced-3 (SEQ ID NO:2) and human interleukin-1β convertase (ICE; SEQ ID NO:4). Vertical bars indicate identical amino acids and single and double dots indicate similar amino acids, where double dots signifies closer similarity than a single dot. The serine-rich region and inactivating mutations of Ced-3 are indicated. The active site and autocleavage sites of ICE are indicated. The portions of the Ced-3 protein encoded by the BGAFQ and PBA constructs are also shown.

Figure 7 shows the alignment of the amino acid sequences of Ced-3 (SEQ ID NO:2) and murine NEDD-2 (SEQ ID NO:13). Vertical bars and single and double dots signify degrees of similarity as in Figures 6A-B. Inactivating mutations of Ced-3 are shown.

Figure 8A shows the alignment of the amino-terminal regions of the Ced-3 proteins of three nematode species (*C. briggsae*, *C. elegans*, and *C. vulgaris*) and mouse (SEQ ID NO:14) and human ICEs. A consensus sequence is also shown. Amino acid positions with the same residue

in more than half of the sequences are shaded. Completely conserved amino acids are also boxed.

Figure 8B shows the alignment of carboxyl-terminal regions of the three nematode Ced-3 proteins, human and mouse ICEs, and the mouse NEDD-2 protein. Except for NEDD-2, these sequences are contiguous with the corresponding sequences shown in Figure 8A. A consensus sequence and amino acid conservation are also shown.

Figure 9 shows a comparison of the Ced-3 proteins of *C. elegans* (line 1; SEQ ID NO:2) and two related nematode species, *C. briggsae* (line 2; SEQ ID NO:5) and *C. vulgaris* (line 3; SEQ ID NO:6). The conserved amino acids are indicated by ".". Gaps inserted in the sequence for the purpose of alignment are indicated by "_".

Figure 10 is the interleukin-1β convertase cDNA sequence (SEQ ID NO:3).

Figure 11A is a schematic representation of two fusion constructs that can prevent programmed cell death.

Figure 11B is a schematic representation of the *lacZ*-containing portion of the fusion constructs.

Detailed Description of the Invention

This invention is based on the discovery that the human enzyme interleukin-1β convertase (ICE) has significant structural similarity to the protein product of the *C. elegans* cell death gene, *ced-3*. The activities of *ced-3* and another cell death gene, *ced-4*, have been shown to be required for almost all the cell deaths which occur during the development of the nematode. ICE is a cysteine protease whose physiological significance has been thought to be related to its role in the maturation of one form of interleukin-1 (IL-1), a major mediator of the immune and

inflammatory response (Fuhlbrigge *et al.*, in: *The Year in Immunology*, Cruse and Lewis (eds.), Karger, Basel, 1989, pp. 21-37). There are two distantly related forms of IL-1, α and β, of which the β form is the predominant species. ICE selectively converts pro-interleukin-1β to the active cytokine, IL-1β. The production of active IL-1β has been implicated in acute and chronic inflammatory diseases, septic shock, and other physiological processes, including wound healing and resistance to viral infection (Ray *et al.*, *Cell 69*:597-604 (1992)). As a result of this discovery, an enzyme which has been known to be involved in the inflammatory response and inflammatory diseases is implicated as having a role in cell death processes. This discovery is consistent with the notion that cell death genes equivalent to the nematode *ced-3* gene function in a variety of organisms. The structural similarity between their gene products suggests that the ICE gene is a human equivalent of the *ced-3* cell death gene. As further described below, the conservation of certain features of ICE in the Ced-3 protein further suggests that Ced-3 is a protease with a substrate-specificity similar to that of ICE.

Furthermore, the identification of *ced-3* and ICE as structurally related genes (i.e., genes whose encoded products, or which themselves, are structurally similar) presents the possibility that a family of structurally related genes exists and provides probes to identify additional members of this *ced-3*/ICE gene family. Comparison of the genes within this family could indicate functionally important features of the genes or their gene products, and thus, provide information for designing drugs which are useful for treating conditions characterized by cell deaths and/or inflammatory disease.

This discovery provides novel drugs based on the *ced-3*, ICE and other *ced-3*/ICE genes and encoded products that inhibit the production of IL-1β and are useful for treatment (preventive and therapeutic) of acute and chronic inflammatory disease, as well as drugs which reduce cell deaths and are useful for treatment of diseases and conditions involving cell deaths (such as myocardial infarction, stroke, traumatic brain injury, viral and other types of pathogenic infection, degenerative diseases, aging, and hair loss). These drugs may also be useful for treating leukemias in which IL-1β has been implicated.

Drugs or agents which increase cell deaths can also be developed based on the *ced-3*, ICE, and related genes and gene products; such drugs or agents may be useful for killing or incapacitating undesired cell populations (such as cancerous cells, infected cells, cells which produce autoreactive antibodies and hair follicle cells) or undesired organisms (such as pests, parasites, and genetically engineered organisms). Drugs are also provided which increase IL-1β production and, therefore, the inflammatory and immune response. These drugs may be helpful for providing increased resistance to viral and other types of infection.

Also described herein is the discovery that fusion constructs containing amino-terminal portions of the *ced-3* gene can inhibit the activity of the intact gene when expressed in otherwise wild-type worms. Due to the similarity between ICE and Ced-3, it is likely that the corresponding amino-terminal portions of the ICE gene will also inhibit the enzymatic activity of ICE in cleaving interleukin-1β. Thus, novel inhibitors of the *ced-3* and ICE genes are provided which may be useful for decreasing cell deaths and/or inflammation involved in various pathologies.

This work has also shown that Ced-3 and the murine NEDD-2 protein are structurally similar. Thus, drugs for increasing or decreasing cell deaths can be developed based on the NEDD-2 gene and its encoded products.

The above-described discoveries, and their implications, and novel drugs and treatments for diseases related to cell death and/or inflammation arising therefrom are described in further detail below.

As used herein, the activity of a gene is intended to include the activity of the gene itself and of the encoded products of the gene. Thus, drugs and mutations which affect the activity of a gene include those which affect the expression as well as the function of the encoded RNA and protein. The drugs may interact with the gene or with the RNA or protein encoded by the gene, or may exert their effect more indirectly.

The *ced-3* Gene

The *C. elegans ced-3* gene was cloned by mapping DNA restriction fragment length polymorphisms (RFLPs) and chromosome walking (Example 1; Figure 1). The gene was localized to a 7.5 kb fragment of cloned genomic DNA by complementation of the *ced-3* mutant phenotype (Figure 2). A 2.8 kb transcript was further identified. The *ced-3* transcript was found to be most abundant in embryos, but was also detected in larvae and young adults, suggesting that *ced-3* is expressed not only in cells undergoing programmed cell death.

A 2.5 kb cDNA corresponding to the *ced-3* mRNA was sequenced. The genomic sequence cloned in the plasmid pJ107 was also determined (Figure 3; SEQ ID NO:1). A comparison with the cDNA sequence revealed that the *ced-3* gene has 7 introns which range in size from 54 to 1195 bp (Figure 4A). The four largest introns, as well as

sequences 5′ of the start codon, contain repetitive elements (Figure 3), some of which have been previously characterized in non-coding regions of other *C. elegans* genes such as *fem-1* (Spence *et al., Cell 60*:981-990 (1990)), *lin-12* (J. Yochem, personal communication), and *myoD* (Krause *et al., Cell 63*:907-919 (1990)). The transcriptional start site was also mapped (Figure 3), and a *ced-3* transcript was found to be trans-spliced to a *C. elegans* splice leader, SL1.

Twelve EMS-induced *ced-3* alleles were also sequenced. Eight of the mutations are missense mutations, three are nonsense mutations, and one is a putative splicing mutation (Table 1). This identification of *ced-3* null alleles, together with results of genetic analysis of nematodes homozygous for these null mutations in *ced-3*, indicate that, like *ced-4, ced-3* function is not essential to viability. In addition, 10 out of the 12 mutations are clustered in the carboxyl-terminal region of the gene (exons 6-8, Figure 4B), suggesting that this portion of the encoded protein may be important for activity.

The *ced-3* gene encodes a putative protein of 503 amino acids (Figure 3; SEQ ID NO:2). The protein is very hydrophilic and no significantly hydrophobic region can be found that might be a transmembrane domain (Figure 5). One region of the Ced-3 protein is very rich in serine (Figures 6A-B). Comparison of the *C. elegans* protein with the Ced-3 proteins of two related nematodes species, *C. briggsae* and *C. vulgaris*, shows conservation of the serine-rich feature without conservation of the amino acid sequence in this region (Figure 9; SEQ ID NO:5-6). This suggests that the exact sequence of this serine-rich region may not be important but that the serine-rich feature is. This hypothesis is supported by analysis of *ced-3* mutations: none of 12

EMS-induced *ced-3* mutations is in the serine-rich region (Figure 4B). It is possible that the serine-rich region in Ced-3 is another example of semi-specific protein-protein interaction, similar to acid blobs in transcription factors and basic residues in nuclear localization signals. In all these cases, the exact primary sequence is not important.

The serine-rich region may function as a site for post-translational regulation of Ced-3 activity through protein phosphorylation of the serine residues by a Ser/Thr kinase. McConkey *et al.* (*J. Immunol*, *145*:1227-1230 (1990)) have shown that phorbol esters, which stimulate protein kinase C, can block the death of cultured thymocytes induced by exposure to Ca^{++} ionophores or glucocorticoids (Wyllie, *Nature 284*:555-556 (1980); Wyllie *et al., J. Path. 142*:67-77 (1984)). It is possible that protein kinase C may inactivate certain cell death proteins by phosphorylation and, thus, inhibit cell death and promote cell proliferation. Several agents that can elevate cytosolic cAMP levels have been shown to induce thymocyte death, suggesting that protein kinase A may also play a role in mediating thymocyte death. Further evidence suggests that abnormal phosphorylation may play a role in the pathogenesis of certain cell-degenerative diseases. For example, abnormal phosphorylation of the microtubule-associated protein Tau is found in the brains of Alzheimer's disease and Down's syndrome patients (Grundke-Iqbal *et al., Proc. Natl. Acad. Sci. USA 83*:4913-4917 (1986); Flament *et al., Brain Res. 516*:15-19 (1990)). Thus, it is possible that phosphorylation may have a role in regulating programmed cell death in *C. elegans*. This is consistent with the fairly high levels of *ced-3* and *ced-4* transcripts which suggest that

transcriptional regulation alone may be insufficient to regulate programmed cell death.

Structural Relatedness of the *ced-3* and Human Interleukin-1β Convertase Genes and Functional Implications

A search of GenBank, PIR and SWISS-PROT databases using the Blast program (National Center for Biotechnology Information) revealed that human interleukin-1β convertase (ICE) has a 28% amino acid identity with the Ced-3 protein (Figures 6A-B). A comparable level of overall similarity was found between ICE and the Ced-3 proteins from two other nematode species, *C. briggsae* and *C. vulgaris*.

The carboxyl-terminal regions of Ced-3 and ICE (amino acids 250-503 and amino acids 166-404, respectively) were found to be more conserved (33% identity) than the amino-terminal portions of the two proteins (22% identity). A comparison of human and murine ICEs also indicated a high degree of similarity (80% identity) in the carboxyl-terminal region compared with an overall identity of 62% (Cerretti *et al.*, *Science 256*:97-100 (1992)). Furthermore, deletion analysis of the ICE cDNA sequence has shown that the amino-terminal 119 amino acids of ICE are not required for enzymatic activity, but that deletions of the carboxyl-terminal region eliminate the enzyme's ability to process pro-IL-1β (Cerretti *et al.*, 1992 *supra*). The observation that most of the inactivating mutations of *ced-3* cluster in the carboxyl-terminal region (Figure 4B) suggests that the activity of Ced-3 also resides (at least partially) in this region. Thus, the identification of the carboxyl-terminal regions of the two proteins as functional domains and the marked similarity of these regions suggest that the Ced-3 and

ICE proteins have similar activities, i.e., that ICE has cell death activity similar to Ced-3 and Ced-3 has protease activity similar to ICE.

The possiblity that Ced-3 has protease activity is further supported by the observation that the region surrounding the active cysteine and two autocleavage sites of ICE appear to be conserved in the Ced-3 protein. As shown in Figures 6A-B, the five amino acids (QACRG, amino acids 283 to 287) surrounding the active cysteine of ICE (Thornberry *et al.*, *Nature 356*:768-774 (1992)) are conserved in amino acids 356 to 360 of Ced-3; this pentapeptide is the longest conserved sequence between ICE and Ced-3. This peptide is also conserved in the Ced-3 proteins of *C. briggsae* and *C. vulgaris* (Figure 9). One inactivating mutation of *ced-3*, *n2433*, introduces a glycine to serine change near the putative active cysteine (Figures 6A-B). The human ICE gene encodes a precursor enzyme which is autoproteolytically cleaved at two major sites (amino acids 103 and 297) by the active form of the enzyme (Thornberry *et al.*, 1992 *supra*). The Asp-Ser dipeptides of both autocleavage sites are conserved in Ced-3 (at amino acids 131 and 371) (Figures 6A-B). The conservation of these functionally important sequences strongly suggests that, like ICE, Ced-3 is a cysteine protease with a similar substrate-specificity. Ced-3 would, therefore, be expected to cleave the IL-1β precursor, as well as other substrates of ICE.

The possibility that ICE is a cell death gene is consistent with evidence which suggests that the production of active IL-1β is involved with cell death processes. Firstly, a variety of studies has suggested that IL-1β can prevent cell death (McConkey *et al.*, *J. Biol. Chem. 265*:3009-3011 (1990); Mangan *et al.*, *J. Immun. 146*:1541-1546 (1991); Sakai *et al.*, *J. Exp. Med.*

166:1597-1602 (1987); Cozzolino *et al.*, *Proc. Natl. Acad. Sci. USA 86*:2369-2373 (1989)). Secondly, active, mature IL-1β appears to be released from cells undergoing cell death. Studies on murine macrophages suggest that release of the active form seems not to be merely due to the lysis of the cells or leaking of cell contents. When murine peritoneal macrophages were stimulated with lipopolysaccharide (LPS) and induced to undergo cell death by exposure to extracellular ATP, mature active IL-1β was released into the culture supernatant. In contrast, when the cells were injured by scraping, IL-1β was released exclusively as the inactive proform (Hogquist *et al.*, *Proc. Natl. Acad. Sci. USA 88*:8485-8489 (1991)).

The similarity between ICE and Ced-3 strongly supports the hypothesis that ICE is involved in cell death. Since Ced-3 is necessary for cell death, one suggestion is that ICE is also necessary for cell death. It is possible that IL-1β can cause cell death. Alternatively, ICE could produce products besides IL-1β, one or more of which can cause cell death. The observation that the ICE transcript is detected in cells that lack IL-1β expression (Cerretti *et al.*, 1992 *supra*) supports this idea.

The finding of a human gene related to the nematode *ced-3* gene is consistent with the idea that cell death genes which are structurally related and/or functionally similar to the nematode *ced-3* gene exist in a variety of organisms. This idea is supported by evidence that cell deaths occurring in a variety of organisms, including vertebrates and invertebrates, and possibly microbes and plants, as well as cell deaths observed in various developmental and pathologic situations share a common genetic mechanism. Evidence for this hypothesis is discussed in Example 2. The structural relatedness of

ICE suggests that it is a mammalian equivalent of the nematode cell death gene, *ced-3*. The cDNA sequence of ICE is shown in Figure 10 (SEQ ID NO:3).

<u>The *ced-3*/ICE Gene Family and Uses Thereof</u>

The ICE and *ced-3* genes can be used to isolate additional structurally related genes, including genes from other organisms. Such genes may be identified using probes derived from both the *ced-3* and ICE gene sequences and known techniques, including nucleic acid hybridization, polymerase chain reaction amplification of DNA, screening of cDNA or genomic libraries, and antibody screening of expression libraries. The probes can be all or portions of the genes which are specific to the genes, RNA encoded by the genes, degenerate oligonucleotides derived from the sequences of the encoded proteins, and antibodies directed against the encoded proteins. The sequences of the genes and their protein products can also be used to screen DNA and protein databases for structurally similar genes or proteins.

One strategy for detecting structurally related genes in a number of organisms is to initially probe animals which are taxonomically closely related to the source of the probes, for example, probing other worms with a *ced-3*-derived probe, or probing other mammals with an ICE-derived probe. Closely related species are more likely to possess related genes or gene products which are detected with the probe than more distantly related organisms. Sequences conserved between *ced-3* or ICE and these new genes can then be used to identify similar genes from less closely related species. Furthermore, these new genes provide additional sequences with which to probe the molecules of other animals, some of which may share conserved regions with

the new genes or gene products but not with the original probe. This strategy of using structurally related genes in taxonomically closer organisms as stepping stones to genes in more distantly related organisms can be referred to as walking along the taxonomic tree.

Together, *ced-3*, ICE, and related genes obtained as described above would comprise a family of structurally related genes, referred to herein as the *ced-3*/ICE gene family. It is highly likely that at least some of these additional family members would exhibit cell death and/or protease activity. The new genes can be tested for protease activity using known assay methods. For example, the sequence of the protein encoded by a new gene may indicate an active site and substrate-specificity similar to that of ICE, such as observed in Ced-3. This activity can then be verified using the transient expression assays and purified enzyme assays previously described (Cerretti *et al.*, *Science 256*:97-100 (1992); Thornberry *et al.*, *Nature 356*:768-774 (1992)). Cell death activity can be tested in bioassays using transgenic nematodes. A candidate cell death gene, such as the ICE gene, can be injected into Ced-3-deficient mutant animals to determine whether the gene complements the *ced-3* mutation. Expression libraries can also be screened for cell death genes by this assay.

The *ced-3*, ICE and other related genes which have cell death activity can be used to develop and identify drugs which reduce or increase cell deaths. Drugs which reduce cell deaths are potentially useful for treating diseases and conditions characterized by cell deaths, such as myocardial infarction, stroke, viral and other pathogenic infections (e.g., human immunodeficiency virus), traumatic brain injury, neural and muscular degenerative diseases, and aging. Drugs which cause cell deaths can be used to control or reduce undesired

cell populations, such as neoplastic growths and other cancerous cells, infected cells, and cells which produce autoreactive antibodies. Undesired organisms, such as pests, parasites, and recombinant organisms, may also be incapacitated or killed by such drugs.

ICE has been implicated in the growth of certain leukemias (*Sakai et al., J. Exp. Med. 166*:1597 (1987); Cozzolino *et al., Proc. Natl. Acad. Sci. U.S.A. 86*:2369 (1989); Estrov *et al., Blood 78*:1476 (1991); Bradbury *et al., Leukemia 4*:44 (1990); Delwel *et al., Blood 74*:586 (1989); Rambaldi *et al., Blood 78*:3248 (1991)). The observation that the human ICE gene maps to chromosome location 11q23, a site frequently involved in DNA rearrangements seen in human cancers (C. *Cerretti et al., Science 256*: 97-100 (1992)), further suggests that ICE is involved in cancer. The finding that ICE probably functions in cell death implies that ICE and other related genes, like *ced-3*, may be used to develop drugs to control cancerous growth.

In addition, since cell death plays an important role in mammalian hair growth, it seems likely that by controlling cell death, one could cause or prevent hair loss. It has been found that *bcl-2*, a human gene which is structurally related to the gene which prevents cell deaths in nematode development (*ced-9*), is expressed in the hair follicle in a cell-cycle dependent manner. *ced-9* has been shown to act by antagonizing the activities of the cell death genes, *ced-3* and *ced-4*. Together, these findings suggest that genes equivalent to the *ced-3*, *ced-4*, and *ced-9* genes are involved in the physiology of mammalian hair growth and loss.

Drugs which increase cell deaths may comprise *ced-3*, ICE, and other *ced-3*/ICE family members, their RNA and protein products, constitutively activated mutants of the genes and encoded products, and peptide

and non-peptide mimetics of the proteins. Drugs which decrease cell deaths may comprise antisense RNA complementary to the mRNA of a cell death gene, or mutant cell death genes or encoded products, that no longer cause cell death and interfere with the function of wild-type genes. Furthermore, drugs comprising agonists and antagonists of the cell death genes can be designed or identified using the genes or their gene products as targets in bioassays. The bioassays can be conducted in wild-type, mutant, or transgenic nematodes, in which an alteration in programmed cell deaths is an indicator of an effective agonist or antagonist. Bioassays can also be performed in cultured cells transfected with the target cell death gene, into which the substance being tested is introduced. In bioassays for antagonists of cell death, the cultured cells should be put under conditions which induce the activity of the target cell death gene.

Uses of bioassays utilizing *C. elegans* are exemplified by the following:

1) use of normal, wild-type nematodes to screen for drugs or genes that inactivate *ced-3* and hence, prevent programmed cell deaths;

2) use of normal, wild-type nematodes to screen for drugs or genes that activate *ced-3* and hence, cause excess cell deaths;

3) use of mutant nematodes which overexpress *ced-3* or which express a constitutively activated *ced-3* gene to identify drugs or genes that prevent excess cell deaths caused by the *ced-3* mutation;

4) use of mutant nematodes which underexpress *ced-3* or which express an inactivated *ced-3* gene to identify drugs or genes that mimic or complement the *ced-3* mutation;

5) use of transgenic nematodes (with an inactivated endogenous *ced-3* gene) in which either a wild-type or mutant form of ICE or other *ced-3*/ICE family member causes excess cell deaths to identify drugs or genes which antagonize the activity of the transgene; and

6) use of transgenic nematodes which carry a transgene that inhibits cell death (e.g., a transgene that expresses an inhibitory fragment of *ced-3*, ICE or related gene, as described below) to identify drugs that overcome this inhibition and cause cell death.

Drugs can be introduced into nematodes by diffusion, ingestion, microinjection, shooting with a particle gun or other methods. They can be obtained from traditional sources such as extracts (e.g., bacterial, fungal or plant) and compound libraries, or can be provided by newer methods of rationale drug design. Information on functionally important regions of the genes or gene products, gained by sequence comparisons and/or mutational analysis may provide a basis for drug design. Genes can be microinjected into nematodes to produce transgenic nematodes. Individual genes or cDNA and genomic DNA libraries can be screened in this manner.

Agonists and antagonists may also be derived from genes which are not cell death genes, but which interact with, regulate or bypass cell death genes. Such interacting genes may be tested by the bioassays mentioned above, as well as by *in vivo* genetics in nematodes. In this latter method, interacting genes are identified as secondary mutations which suppress or enhance the *ced-3* mutation. The sequences of these interacting genes can then be used to identify structurally related interacting genes in other organisms.

Similarly, anti-inflammatory drugs may be developed or identified using *ced-3*, ICE and other family members and their encoded products. Drugs which enhance ICE activity may also be useful for boosting the inflammatory response to viral and other infections.

In addition, the availability of a number of structurally related genes makes it possible to carry out structural comparisons. Conserved regions or features of the genes or their encoded products are likely to be functionally significant for cell death and/or protease activity. This information could be helpful in designing or selecting drugs which would mimic or affect the activity of the genes.

Moreover, conservation of functional domains among *ced-3*/ICE family members or their encoded products suggests not only that these genes have similar activities, but that they and their encoded products function via similar mechanisms. This suggests that mutations in conserved regions, mimetics based on conserved regions, and agonists and antagonists which affect the function of conserved regions of one *ced-3*/ICE gene or encoded protein will similarly affect other genes or encoded proteins in the family. This is the rationale behind the use of Ced-3 inhibitors to inhibit ICE and inflammation, and the use of anti-inflammatory drugs which act by inhibiting ICE to inhibit the *ced-3* gene and reduce cell deaths (described further below).

Furthermore, drugs which affect the cell death and/or inflammatory activities of the *ced-3* and ICE genes may also affect other as yet undiscovered activities of these genes. The biology of IL-1β and ICE is only incompletely understood at the present time, and it is very likely that other functions of both IL-1β and ICE may be discovered. These may include new activities

or new physiological processes or diseases in which the respective cytokinetic and proteolytic activities of these molecules are involved. In either case, drugs (such as inhibitory protein portions) which affect ICE activity are likely to affect the new activities and processes, as well.

In addition, mutations and drugs which alter or mimic the activity of one member of the *ced-3*/ICE family can be engineered based on what is known about mutations and drugs affecting another family member with which it shares a conserved region. Mutations in conserved regions which correspond to those found in another family member could be used to produce similar effects. For example, five out of nine inactivating point mutations analyzed in *ced-3* were found to result in alterations of amino acids which are conserved between ICE and Ced-3 (Figures 6A-B). Amino acid substitutions in ICE corresponding to those in Ced-3 are also expected to result in inactivation. The inhibitory amino-terminal gene portions and constitutively activated carboxyl-terminal gene portions described below are further examples of corresponding mutations which can be made in genes of the *ced-3*/ICE family.

Comparison of Ced-3, ICE, and related proteins may also provide insights into the substrate-specificity of ICE and related enzymes. Previous studies on ICE have not identified a consistent consensus cleavage site. A comparison of the Ced-3 and ICE autocleavage sites, together with the cleavage site of pro-IL-1β, reveals that cleavage always occurs after an Asp residue. For this reason, it is likely that Ced-3, ICE, and related proteins are proteases which cleave after some aspartate residues or, perhaps at lower efficiencies, all aspartate residues.

A further use of *ced-3*/ICE family members is to provide diagnostic probes (DNA, RNA, oligonucleotides and antibodies) for diseases involving cell deaths and inflammation in humans and other organisms. It is likely that such diseases are associated with abnormalities in *ced-3*/ICE genes and their gene products. The probes can be used to detect abnormalities in the sequence, level and/or activities of the genes and encoded RNA and protein products. The diseases may be genetic, in which case, the probes may be used in patient and pre-natal testing, or non-genetic, in which case, RNAs and proteins may be examined. In particular, the finding that ICE is a putative cell death gene makes this gene and its derivative molecules potentially useful as diagnostic probes for diseases characterized by cell deaths. Similarly, *ced-3* and its derivative molecules are potentially useful for detecting abnormalities in pathologies in which inflammation is evident. The usefulness of these probes may be multiplied as more genes with known physiological functions are found to be structurally related to *ced-3* and ICE.

Structural Relatedness of *ced-3* and the Murine NEDD-2 Gene

Database searches also revealed that another mammalian protein is similar to the Ced-3 protein (Figure 7). The murine NEDD-2 protein has 27% amino acid identity and 55% similarity to a carboxyl-terminal portion of Ced-3. The NEDD-2 protein is expressed in the brain of mouse embryos and much less in the murine adult brain; the protein is thought to be involved in the development of the murine central nervous system (Kumar *et al.*, *Biochem. Biophys. Res. Comm.* *185*(3):1155-1161 (1992)). The structural similarity between the

NEDD-2 and *ced-3* gene products suggests that the NEDD-2 gene is also involved in cell death processes which occur during development, and further supports the hypothesis that genes which are structurally and functionally related to the nematode *ced-3* gene function in a variety of organisms. Interestingly, the NEDD-2 amino acid sequence is not significantly similar to that of human ICE.

The similarity of the amino acid sequences of Ced-3 and NEDD-2 further suggests that mutations of the NEDD-2 gene which produce alterations in the protein corresponding to alterations in Ced-3 resulting from the mutations, *n1129*, *n1164*, *n2426* and *n1163* (see Figure 7), will inactivate the NEDD-2 gene.

This invention includes all and portions of the NEDD-2 gene, mutated NEDD-2 genes corresponding to known *ced-3* mutations, RNAs and proteins encoded by the wild-type and mutated genes, and mimetics and other drugs derived from these genes and gene products, which are useful for controlling cell death.

Figures 8A and 8B show alignments of the amino-terminal and carboxyl-terminal regions, respectively, of the Ced-3 proteins of the three nematode species (*C. briggsae*, *C. elegans*, and *C. vulgaris*), the human and murine ICEs and the murine NEDD-2 protein (in 8B only). As shown in these figures (boxed portions), a number of amino acids are completely conserved among these structurally related proteins, and thus, are likely to be important functionally. Mutations of these sites would be expected to alter the activity of the genes.

Inhibitory Portions of the *ced-3* Gene

Fusion constructs containing portions of the *ced-3* gene were found to prevent programmed cell death when expressed in wild-type *C. elegans*. These constructs are

represented schematically in Figure 11A. The BGAFQ construct contains a portion of the *ced-3* gene fused 5' of the *E. coli lacZ* gene and another *ced-3* portion fused 3' of *lacZ*. The 5' *ced-3* portion is the genomic sequence from a *Bam*HI site located about 300 base pairs upstream of nucleotide 1 of the sequence shown in Figure 3 to a *Sal*I site at nucleotide 5850. This portion spans sequences 5' of the SL1 acceptor site (nucleotide 2161) to include the 372 codons of the amino-terminal region. The 3' *ced-3* portion of BGAFQ is the genomic sequence from a *Not*I site at nucleotide 5927 in the *ced-3* gene to an *Apa*I site located about 1.5 kb downstream of nucleotide 7653 of the sequence in Figure 3. This portion contains the carboxyl-terminal codons from 398 to the end (codon 503) and 3' untranslated sequences.

The PBA construct has a smaller portion of the *ced-3* gene which is the genomic sequence from the same *Bam*HI site as in BGAFQ to a *Bgl*II site at nucleotide 3020 (Figure 11A) fused 5' of the *lacZ* gene. This *ced-3* portion spans sequences 5' of the SL1 acceptor site to include the first 149 codons of the amino-terminal region.

Both constructs were made using the pBluescript vector (Stratagene) and fragments containing the *lacZ* construct from the pPD vectors of Fire (*EMBO J. 5*:2673-2690 (1986)). The *lacZ*-containing portion has the entire *lacZ* coding sequence except for the first 11 codons. In addition, there is a synthetic intron and a nuclear localization signal upstream of the *lacZ* gene and a fragment of the 3' end of the *unc-54* gene downstream of the *lacZ* gene (Figure 11B). Construct PBA was made by inserting a *Bam*HI-*Apa*I fragment containing the *lacZ* construct shown in Figure 11B from Andy Fire's vector, pPD22.04, into the *Bgl*II-*Apa*I fragment of the *ced-3*-containing plasmid, pJ40. Construct BGAFQ was

made by inserting a *Sal*I-*Eag*I fragment containing the same *lacZ* construct from pPD22.04 into the *Sal*I-*Not*I fragment of pJ40A, which is pJ40 without the *Not*I site in the vector.

Table 2 shows the results of injecting wild-type nematodes with the two constructs. These results indicate that the BGAFQ and PBA fusion constructs prevent cell deaths which normally occur in the development of the nematodes. These fusion constructs were further observed to prevent cell deaths and the apparently associated inviability caused by a loss-of-function mutation in *ced-9*, a gene which functions to keep certain cells from dying during nematode development, and which has been shown to act by antagonizing *ced-3* and second cell death gene, *ced-4*.

Both constructs express β-galactosidase activity in wild-type nematodes. Since the pBluescript vector does not contain eukaryotic transcriptional or translational start sites, these signals are probably supplied by the *ced-3* gene portions fused 5′ of *lacZ*. Furthermore, since the PBA construct works to prevent cell death, it seems that the *ced-3* portion in BGAFQ needed for inhibition is the portion fused upstream of *lacZ* (as opposed to the portion located downstream of *lacZ*). Presumably, only the region from the *Bam*HI site to nucleotide 3020 is needed in BFAGQ, since this is all that is contained in PBA.

A construct that contains the PBA *ced-3* portion but not any of the *lacZ* portion did not prevent cell death, suggesting that fusion to portions of *lacZ* is needed for expression or action of the inhibitory gene portion.

These observations indicate that the amino-terminal portion of the Ced-3 protein, possibly in conjunction with a portion of *E. coli* β-galactosidase, can act to prevent programmed cell deaths in *C. elegans*. One

plausible mechanism is that this portion of the Ced-3 protein acts in a dominant negative or antimorphic fashion, to prevent the activity of the normal Ced-3 protein. (It is known that inactivation of the Ced-3 protein results in an absence of programmed cell deaths.) Such dominant negative activity could be a result of the partial Ced-3 protein binding to and, thereby, inactivating the normal Ced-3 protein; consistent with this model is the finding that the active form of the structurally similar ICE protein is dimeric. Alternatively, the partial Ced-3 protein may bind to a molecule with which the normal Ced-3 protein must interact to function and by preventing this interaction, inhibits Ced-3 activity.

Due to the structural similarity of ICE to the Ced-3 protein, fusion constructs encoding amino-terminal portions of ICE would also be expected to inhibit the activity of the *ced-3* gene. In particular, those portions of the ICE gene corresponding to the *ced-3* gene portions in BGAFQ and PBA, i.e., ICE codons 1 to 298 and codons 1 to 111, or active subportions of these, are expected to inhibit *ced-3*. A further extension of this reasoning suggests that corresponding gene portions of any structurally related *ced-3*/ICE family member would also have an inhibitory effect on *ced-3* activity.

Furthermore, the structural relatedness of the *ced-3* and ICE genes implies that the ICE enzyme could also be inhibited by fusion constructs containing amino-terminal portions of the ICE gene, as well as corresponding portions of other structurally related genes, such as *ced-3*.

Identification of portions of the *ced-3*, ICE, and related genes which inhibit the *ced-3* gene can be carried out by testing expression constructs containing these gene portions or their encoded products in

bioassays for cell death activity. Identification of gene portions or encoded products which inhibit ICE can be carried out using previously described assays for ICE activity. For example: 1) wild-type worms can be injected with portions of the *ced-3* or other structurally related gene, such as ICE, to determine if they prevent programmed cell death; 2) portions of the ICE protein or other structurally similar protein, such as Ced-3, can be co-expressed with ICE and pro-IL-1β in nematodes or cultured mammalian cells to see if they inhibit ICE-catalyzed cleavage of the IL-1β precursor; and 3) peptides or nucleic acids containing portions of the amino acid or coding sequence of ICE or similar protein, such as Ced-3, can be tested using purified ICE and synthetic substrates.

Inhibitory portions of the *ced-3* gene, ICE, and structurally related genes, their encoded RNAs and proteins, and peptide and non-peptide mimetics of the proteins may be used to reduce cell deaths and/or inflammation, and are, thus, useful for the treatment of diseases involving these processes. The encoded proteins and peptide and non-peptide mimetics can be delivered by various known methods and routes of drug delivery. For example, they can be administered orally or by another parenteral route or by a non-parenteral route (e.g., by injection intramuscularly, intraperitoneally or intravenously or by topical administration). Alternatively, expression constructs containing the gene portions can be made using heterologous transcriptional and translational signals or signals native to the gene portions. The constructs can be delivered into cells by various methods of gene therapy, such as retroviral infection.

Interestingly, those ICE gene portions corresponding to the *ced-3* portions of BGAFQ and PBA

encode approximately the protein fragments which result from cleavage at each of the two autocleavage sites (amino acids 103 and 297). This observation suggests that autoproteolytic conversion of the proenzyme to active ICE involves cleaving off the inhibitory amino-terminal portions of the protein. Active ICE is a heterodimer composed of subunits of about 20 and 10 kilodaltons (Thornberry *et al.*, *Nature 356*:768-774 (1992)). These subunits have been shown to be derived from the ICE proenzyme and correspond to amino acids 120 to 297 (p20) and 317 to 404 (p10). Kinetic studies suggest that association of the two subunits is required for activity of the enzyme. It is possible that the amino-terminal region of the protein interferes with this association.

This implies that mutant proteins in which the inhibitory amino-terminal regions are deleted may be constitutively activated. Thus, carboxyl-terminal portions of the Ced-3, ICE, and related proteins, and constructs and RNAs expressing these portions, are potentially useful for increasing cell deaths and/or IL-1β production. Constructs which may be used include those which express the carboxyl region of ICE, which encodes the two subunits of the active enzyme, as well as those which express each of these subunits separately. In addition, it is possible that the amino region of ICE, which is not needed for ICE enzymatic activity *in vitro*, is important for ICE activity or the regulation of ICE activity *in vivo*. Consistent with this idea is the finding that two of the *ced-3* mutations map in this region. For this reason, a construct which expresses the amino region of Ced-3, ICE or a Ced-3/ICE gene family member may also be used. Furthermore, the NEDD-2 protein, which is similar to a carboxyl-terminal portion of the Ced-3 portion, may also exhibit

constitutive activity in causing cell deaths. Thus, all or active portions of NEDD-2, and DNA and RNA encoding NEDD-2 proteins, would be expected to produce cell death activity when expressed. Drugs comprising activated molecules derived from the carboxyl-terminal regions of Ced-3, ICE and other proteins of the Ced-3/ICE family and from the NEDD-2 protein, DNAs and RNAs encoding these proteins and protein fragments, as well as peptide and non-peptide mimetics, are potentially useful for controlling or reducing the size of undesirable cell populations, such as cancerous cells, infected cells, cells producing autoreactive antibodies and hair follicle cells. Such drugs may also be useful for incapacitating or killing undesired organisms, such as parasites, pests, and genetically engineered organisms. For example, a number of nematodes are human, animal and plant parasites.

<u>ICE Inhibitors As Inhibitors of Cell Death</u>

The conservation of the active site of ICE (active cysteine and surrounding amino acids) in the Ced-3 protein implies that Ced-3 is a cysteine protease which interacts with its substrate by a similar mechanism. Hence, it is likely that inhibitors of ICE which interfere with this mechanism, or chemical analogs of these inhibitors, will also inhibit Ced-3 function.

Peptide aldehydes containing the ICE recognition site:

P4--P3--P2--P1
Tyr-Val-Ala-Asp

or a substituted site in which P2 is Ala, His, Gln, Lys, Phe, Cha, or Asp, have been shown to be effective, specific, and reversible inhibitors of the protease activity of ICE (Thornberry *et al.*, *Nature 356*:768-774

(1992)). These molecules are thought to act as transition analogs, which compete for ICE binding to its substrate, pro-IL-1β. Three such inhibitors have been described: Inhibitor B (Ac-Tyr-Val-Ala-Asp-CHO); Inhibitor C (Ac-Tyr-D-Ala-Ala-Asp-CHO); and Inhibitor D (Ac-Tyr-Val-Lys-Asp-CHO). Of these, Inhibitor B is the most potent, with a K_i=0.76 nM compared to K_i=3 nM for D and K_i=1.5 μM for C.

In addition, the *crmA* gene of cowpox virus has been found to encode a serpin which specifically inhibits ICE (Ray *et al.*, *Cell 69*:597-604 (1992)). The serpin acts by preventing the proteolytic activation of ICE. This inhibitor of ICE is also expected to inhibit structurally similar proteins, such as Ced-3. The *crmA* gene and methods for obtaining purified CrmA protein have been described (Pickup *et al.*, *Proc. Natl. Acad. Sci. USA 83*:7698-7702 (1986); Ray *et al.*, 1992 *supra*). This invention includes the use of inhibitors of ICE, such as peptide aldehydes, and particularly inhibitor B, and the CrmA protein, as drugs for decreasing the activity of cell death genes and, thus, for treatment of diseases characterized by cell deaths.

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims. For example, functional equivalents of DNAs and RNAs may be nucleic acid sequences which, through the degeneracy of the genetic code, encode the same proteins as those specifically claimed. Functional equivalents of proteins may be substituted or modified amino acid sequences, wherein the substitution or modification does not change the activity or function of the protein. A

"silent" amino acid substitution, such that a chemically similar amino acid (e.g., an acidic amino acid with another acidic amino acid) is substituted, is an example of how a functional equivalent of a protein can be produced. Functional equivalents of nucleic acids or proteins may also be produced by deletion of nonessential sequences.

The following examples illustrate the invention and are not intended to be limiting in any way.

EXAMPLE 1

CLONING, SEQUENCING, AND CHARACTERIZATION OF THE *CED-3* GENE

MATERIALS AND METHODS

General Methods and Strains

The techniques used for the culturing of *C. elegans* were as described by Brenner (*Genetics* 77:71-94 (1974)). All strains were grown at 20°C. The wild- type parent strains were *C. elegans* variety Bristol strain N2, Bergerac strain EM1002 (Emmons *et al.*, *Cell* 32:55-65 (1983)), *C. briggsae* and *C. vulgaris* (obtained from V. Ambros). The genetic markers used are described below. These markers have been described by Brenner (1974 *supra*), and Hodgkin *et al.* (In: *The Nematode Caenorhabditis elegans*, Wood and the Community of *C. elegans* Researchers (eds.), Cold Spring Harbor Laboratory, 1988, pp 491-584). Genetic nomenclature follows the standard system (Horvitz *et al.*, *Mol. Gen. Genet.* *175*:129-133 (1979)):

LG I: *ced-1(e1375); unc-54(r323)*
LG VI: *unc-31(e928), unc-30(e191), ced-3(n717, n718, n1040, n1129, n1163, n1164, n1165, n1286,*

n1949, n2426, n2430, n2433), unc-26(e205), dpy-4(e1166)

LG V: *egl-1(n986); unc-76(e911)*

LG X: *dpy-3(e27)*

<u>Isolation of Additional Alleles of *ced-3*</u>

A non-complementation screen was designed to isolate new alleles of *ced-3*. Because animals heterozygous for *ced-3(n717)* in *trans* to a deficiency are viable (Ellis and Horvitz, *Cell 44*:817-829 (1986)), animals carrying a complete loss-of-function *ced-3* allele generated by mutagenesis were expected to be viable *in trans* to *ced-3(n717)*, even if the new allele was inviable in homozygotes. Fourteen EMS mutagenized *egl-1* males were mated with *ced-3(n717) unc-26(e205); egl-1(n487); dpy-3(e27)* hermaphrodites. *egl-1* was used as a marker in this screen. Dominant mutations in *egl-1* cause the two hermaphrodite specific neurons, the HSNs, to undergo programmed cell death (Trent *et al.*, *Genetics 104*:619-647 (1983)). The HSNs are required for normal egg-laying, and egl-1(n986) hermaphrodites, which lack HSNs, are egg-laying defective (Trent *et al.*, 1983 *supra*). The mutant phenotype of *egl-1* is suppressed in a *ced-3; egl-1* strain because mutations in *ced-3* block programmed cell deaths. *egl-1* males were mutagenized with EMS and crossed with *ced-3(n717), unc-26(e205); egl-1(n487); dpy-3(e27)*. Most cross progeny were egg-laying defective because they were heterozygous for *ced-3* and homozygous for *egl-1*. Rare egg-laying competent animals were picked as candidates for carrying new alleles of *ced-3*. Four such animals were isolated from about 10,000 F1 cross progeny of EMS-mutagenized animals. These new mutations were made homozygous to confirm that they carried recessive mutations of *ced-3*.

Molecular Biology

Standard techniques of molecular biology were used (Maniatis *et al.*, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, 1983).

Two cosmid libraries were used extensively in this work: a *Sau*3AI partial digest genomic library of 7000 clones in the vector pHC79 and a *Sau*3AI partial digest genomic library of 6000 clones in the vector pJB8 (Ish-Horowicz and Burke, *Nucleic Acids Res*. *9*:2989 (1981)).

The "right" end of MMM-C1 was cloned by cutting it with *Hin*dIII and self-ligating. The "left" end of MMM-C1 was cloned by cutting it with *Bgl*II or *Sal*I and self-ligating.

The "right" end of Jc8 was made by digesting Jc8 with *Eco*RI and self-ligating. The "left" end of Jc8 was made by digesting Jc8 by *Sal*I and self-ligating.

C. elegans RNA was extracted using guanidine isothiocyanate (Kim and Horvitz, *Genes & Dev*. *4*:357-371 (1990)). Poly$(A)^+$ RNA was selected from total RNA by a poly(dT) column (Maniatis et al., 1983 *supra*). To prepare stage-synchronized animals, worms were synchronized at different developmental stages (Meyer and Casson, *Genetics 106:29-44* (1986)).

For DNA sequencing, serial deletions were made according to a procedure developed by Henikoff (*Gene 28*:351-359 (1984)). DNA sequences were determined using Sequenase and protocols obtained from US Biochemicals with minor modifications.

The Tc1 DNA probe for Southern blots was pCe2001, which contains a Bergerac Tc1 element (Emmons *et al.*, *Cell 32*:55-65 (1983)). Enzymes were purchased from New England Biolabs, and radioactive nucleotides were from Amersham.

Primer extension procedures followed the pro by Robert E. Kingston (In: *Current Protocols in Molecular Biology*, Ausubel *et al.* (eds.), Greene Publishing Associates and Wiley-Interscience, Ne p. 4.8.1) with minor modifications.

Polymerase chain reaction (PCR) was carried o using standard protocols supplied by the GeneAmp Kit (Perkin Elmer). The primers used for primer extension and PCR are as follows:

Pex2: 5' TCATCGACTTTTAGATGACTAGAGAACATC 3' (SEQ ID NO:7);
Pex1: 5' GTTGCACTGCTTTCACGATCTCCCGTCTCT 3' (SEQ ID NO:8);
SL1: 5' GTTTAATTACCCAAGTTTGAG 3' (SEQ ID NO:9);
SL2: 5' GGTTTTAACCAGTTACTCAAG 3' (SEQ ID NO:10);
Log5: 5' CCGGTGACATTGGACACTC 3' (SEQ ID NO:11); and
Oligo10: 5' ACTATTCAACACTTG 3' (SEQ ID NO:12).

<u>Germline Transformation</u>

The procedure for microinjection basically follows that of A. Fire (*EMBO J.* *5*:2673-2680 (1986)) with modifications: Cosmid DNA was twice purified by CsCl-gradient. Miniprep DNA was used when deleted cosmids were injected. To prepare miniprep DNA, DNA from 1.5 ml overnight bacterial culture in superbroth (12 g Bacto-tryptone, 24 g yeast extract, 8 ml 50% glycerol, 900 ml H_2O, autoclaved; after autoclaving, 100 ml 0.17 M KH_2PO_4 and 0.72 M KH_2PO_4 were added) was extracted by alkaline lysis method as described in Maniatis et al. (1983 *supra*). DNA was treated with RNase A (37°, 30 minutes) and then with protease K (55°, 30 minutes), extracted with phenol and then chloroform, precipitated twice (first in 0.3 M sodium acetate and second in 0.1 M

potassium acetate, pH 7.2), and resuspended in 5 μl injection buffer as described by A. Fire (1986 *supra*). The DNA concentration for injection is in the range of .00 ug to 1 mg per ml.

All transformation experiments used *ced-1(e1735); unc-31(e928) ced-3(n717)* strain. *unc-31* was used as a marker for co-transformation (Kim and Horvitz, 1990 *supra*). *ced-1* was present to facilitate scoring of the Ced-3 phenotype. The mutations in *ced-1* block the engulfment process of cell death, which makes the corpses of the dead cells persist much longer than in wild-type animals (Hedgecock *et al.*, *Science 220*:1277-1280 (1983)). The Ced-3 phenotype was scored as the number of dead cells present in the head of young L1 animals. The cosmid C10D8 or the plasmid subclones of C10D8 were mixed with C14G10 (unc-31(+)-containing) at a ratio of 2:1 or 3:1 to increase the chances that a Unc-31(+) transformant would contain the cosmid or plasmid being tested as well. Usually, 20-30 animals were injected in one experiment. Non-Unc F1 progeny of the injected animal were isolated three to four days later. About 1/2 to 1/3 of the non-Unc progeny transmitted the non-Unc phenotype to F2 progeny and established a transformant line. The young L1 progeny of such non-Unc transformant were checked for the number of dead cells present in the head using Nomarski optics.

RESULTS

Isolation of Additional *ced-3* Alleles

All of the *ced-3* alleles that existed previously were isolated in screens designed to detect viable mutants displaying the Ced phenotype (Ellis and Horvitz, *Cell 44*:817-829 (1986)). Such screens may have systematically missed any class of *ced-3* mutations that

is inviable as homozygotes. For this reason, a scheme was designed that could isolate recessive lethal alleles of *ced-3*. Four new alleles of *ced-3 (n1163, n1164, n1165, n1286)* were isolated in this way. Since new alleles were isolated at a frequency of about 1 in 2500, close to the frequency expected for the generation of null mutations by EMS in an average *C. elegans* gene (Brenner, *Genetics 77*:71-94 (1974); Greenwald and Horvitz, *Genetics 96*:147-160 (1980)), and all four alleles are homozygous viable, it was concluded that the null allele of *ced-3* is viable.

Mapping RFLPs near *ced-3*

Tc1 is a *C. elegans* transposable element that is thought to be immobile in the common laboratory Bristol strain and in the Bergerac strain (Emmons *et al.*, *Cell 32*:55-65 (1983)). In the Bristol strain, there are 30 copies of Tc1, while in the Bergerac strain, there are more than 400 copies of Tc1 (Emmons et al., 1983 *supra*; Finney, Ph.D. thesis, Massachusetts Institute of Technology, Cambridge, Massachusetts, 1987). Because the size of the *C. elegans* genome is small (haploid genome size 8 x 10^7 bp) (Sulston and Brenner, *Genetics 77*:95-104 (1976)), a polymorphism due to Tc1 between the Bristol and Bergerac strains would be expected to occur about once every 200 kb. Restriction fragment length polymorphisms (RFLPs) can be used as genetic markers and mapped in a manner identical to conventional mutant phenotypes. A general scheme has been designed to map Tc1 elements that are dimorphic between the Bristol and Bergerac strains near any gene of interest (Ruvkun *et al.*, *Genetics 121*:501-516 (1989)). Once tight linkage of a particular Tc1 to a gene of interest has been

established, that Tc1 can be cloned and used to initiate chromosome walking.

A 5.1 kb Bristol-specific Tc1 *Eco*RI fragment was tentatively identified as containing the Tc1 closest to *ced-3*. This Tc1 fragment was cloned using cosmids from a set of Tc1-containing *C. elegans* Bristol genomic DNA fragments. DNA was prepared from 46 such Tc1-containing cosmids and screened using Southern blots to identify the cosmids that contain a 5.1 kb *Eco*RI Tc1-containing fragment. Two such cosmids were identified: MMM-C1 and MMM-C9. The 5.1 kb *Eco*RI fragment was subcloned from MMM-C1 into pUC13 (Promega). Since both ends of Tc1 contain an *Eco*RV site (Rosenzweig *et al.*, *Nucleic Acids Res.* *11*:4201-4209 (1983)), *Eco*RV was used to remove Tc1 from the 5.1 kb *Eco*RI fragment, generating a plasmid that contains only the unique flanking region of this Tc1-containing fragment. This plasmid was then used to map the specific Tc1 without the interference of other Tc1 elements.

unc-30(e191) ced-3(n717) dpy-4(e1166)/+++ males were crossed with Bergerac (EM1002) hermaphrodites, and Unc non-Dpy or Dpy non-Unc recombinants were picked from among the F2 progeny. The recombinants were allowed to self-fertilize, and strains that were homozygous for either *unc-30(e191) dpy-4(Bergerac)* or *unc-30(Bergerac) dpy-4(e1166)* were isolated. After identifying the *ced* genotypes of these recombinant strains, DNA was prepared from these strains. A Southern blot of DNA from these recombinants was probed with the flanking sequence of the 5.1 kb *Eco*RI Tc1 fragment. This probe detects a 5.1 kb fragment in Bristol N2 and a 3.4 kb fragment in Bergerac. Five out of five *unc-30 ced-3 dpy(+Berg)* recombinants, and one of one *unc-30(+Berg) ced-3 dpy-4* recombinants showed the Bristol pattern. Nine of ten *unc-30(+Berg) dpy-4* recombinants showed the Bergerac

pattern. Only one recombinant of *unc-30(+Berg) dpy-4* resulted from a cross-over between *ced-3* and the 5.1 kb Tc1 element. The genetic distance between *ced-3* and *dpy-4* is 2 map units (mu). Thus, this Tc1 element is located 0.1 mu on the right side of *ced*-3.

Cosmids MMM-C1 and MMM-C9 were used to test whether any previously mapped genomic DNA cosmids overlapped with these two cosmids. A contig of overlapping cosmids was identified that extended the cloned region near *ced-3* in one direction.

To orient MMM-C1 with respect to this contig, both ends of MMM-C1 were subcloned and these subclones were used to probe the nearest neighboring cosmid C48D1. The "right" end of MMM-C1 does not hybridize to C48D1, while the "left" end does. Therefore, the "right" end of MMM-C1 extends further away from the contig. To extend this contig, the "right" end of MMM-C1 was used to probe the filters of two cosmid libraries (Coulson *et al.*, *Proc. Natl. Acad. Sci. USA 83*:7821-7825 (1986)). One clone, Jc8, was found to extend MMM-C1 in the opposite direction of the contig.

RFLPs between the Bergerac and Bristol strains were used to orient the contig with respect to the genetic map. Bristol (N2) and Bergerac (EM1002) DNA was digested with various restriction enzymes and probed with different cosmids to look for RFLPs. Once such an RFLP was found, DNA from recombinants of the Bristol and Bergerac strains between *ced-3* and *unc-26*, and between *unc-30* and *ced-3* was used to determine the position of the RFLP with respect to *ced-3*.

The "right" end of Jc8, which represents one end of the contig, detects an RFLP *(nP33)* when N2 and EM1002 DNA was digested with *Hin*dIII. A Southern blot of DNA from recombinants between three *ced-3(+Berg) unc-26* was probed with the "right" end of Jc8. Three of three

+Berg unc-26 recombinants showed the Bristol pattern, while two of two *ced-3 unc-26(+Berg)* recombinants showed the Bergerac pattern. Thus, *nP33* mapped very close or to the right side of *unc-26*.

The "left" end of Jc8 also detects a *Hin*dIII RFLP *(nP34)*. The same Southern blot was reprobed with the Jc8 "left" end. Two of the two *ced-3 unc-26(+Berg)* recombinants and two of the three *ced-3(+Berg) unc-26* recombinants showed the Bergerac pattern. One of the three *ced-3(+Berg) unc-26* recombinants showed the Bristol pattern. The genetic distance between *ced-3* and *unc-26* is 0.2 mu. Thus, *nP34* was mapped between *ced-3* and *unc-26*, about 0.1 mu on the right side of *ced-3*.

The flanking sequence of the 5.1 kb *Eco*RI Tc1 fragment (named *nP35*) was used to probe the same set of recombinants. Two of three *ced-3(+Berg) unc-26* recombinants and two of two *ced-3 unc-26(+Berg)* recombinants showed the Bristol pattern. Thus, *nP35* was also found to be located between *ced-3* and *unc-26*, about 0.1 mu on the right side of *ced-3*.

A similar analysis using cosmid T10H5 which contains the *Hin*dIII RFLP *(nP36)*, and cosmid B0564, which contains a *Hin*dIII RFLP (*nP37*), showed that *nP36* and *nP37* mapped very close or to the right of *unc-30*.

These experiments localized the *ced-3* gene to an interval of three cosmids. The positions of the RFLPs, and of *ced-3, unc-30* and *unc-26* on chromosome IV, and their relationships to the cosmids are shown in Figure 1. It has been demonstrated by microinjection that cosmids C37G8 and C33F2 carry the *unc-30* gene (John Sulston, personal communication). Thus, the region containing the *ced-3* gene was limited to an interval of two cosmids. These results are summarized in Figure 1.

<u>Complementation of *ced-3* by Germline Transformation</u>

Cosmids that were candidates for containing the ced-3 gene were microinjected into a *ced-3* mutant to see if they rescue the mutant phenotype. The procedure for microinjection was that of A. Fire (*EMBO J. 5*:2673-2680 (1986)) with modifications. *unc-31*, a mutant defective in locomotion, was used as a marker for cotransformation (Kim and Horvitz, *Genes & Dev. 4*:357-371 (1990)), because the phenotype of *ced-3* can be examined only by using Nomarski optics. Cosmid C14G10 (containing *unc-31*(+)) and a candidate cosmid were coinjected into *ced-1(e1375); unc-31(e928) ced-3(n717)* hermaphrodites, and F1 non-Unc transformants were isolated to see if the non-Unc phenotype could be transmitted and established as a line of transformants. Young L1 progeny of such transformants were examined for the presence of cell deaths using Nomarski optics to see whether the Ced-3 phenotype was suppressed. Cosmid C14G10 containing *unc-31* alone does not rescue *ced-3* activity when injected into a *ced-3* mutant. Table 4 summarizes the results of these transformation experiments.

As shown in Table 3, of the three cosmids injected (C43C9, W07H6 and C48D1), only C48D1 rescued the Ced-3 phenotype (2/2 non-Unc transformants rescued the Ced-3 phenotype). One of the transformants, *nEX2*, appears to be rescued by an extra-chromosomal array of injected cosmids (Way and Chalfie, *Cell 54*:5-16 (1988)), which is maintained as an unstable duplication, since only 50% of the progeny of a non-Unc Ced(+) animal are non-Unc Ced(+). Since the non-Unc Ced(+) phenotype of the other transformant (nIS1) is transmitted to all of its progeny, it is presumably an integrated transformant. L1 *ced-1* animals contain an average of 23 cell corpses in the head. L1 *ced-1; ced-3* animals contain an average of 0.3 cell corpses in the head. *ced-1; unc-31 ced-3; nIS1* and *ced-1; unc-31 ced-3; nEX2* animals contain an

average of 16.4 and 14.5 cell corpses in the head, respectively. From these results, it was concluded that C48D1 contains the *ced-3* gene.

In order to locate *ced-3* more precisely within the cosmid C48D1, this cosmid was subcloned and the subclones were tested for the ability to rescue *ced-3* mutants. C48D1 DNA was digested with restriction enzymes that cut rarely within the cosmid and the remaining cosmid was self-ligated to generate a subclone. Such subclones were then injected into a *ced-3* mutant to look for completion. When C48D1 was digested with *Bam*HI and self-ligated, the remaining 14 kb subclone (named C48D1-28) was found to rescue the Ced-3 phenotype when injected into a *ced-3* mutant (Figure 2 and Table 4). C48D1-28 was then partially digested with *Bgl*II and self-ligated. Clones of various lengths were isolated and tested for their ability to rescue *ced-3*.

One clone, C48D1-43, which did not contain a 1.7 kb *Bgl*II fragment of C48D1-28, was able to rescue *ced-3* (Figure 2 and Table 4). C48D1-43 was further subcloned by digesting with *BamHI* and *ApaI* to isolate a 10 kb *Bam*HI-*Apa*I fragment. This fragment was subcloned into pBSKII+ to generate pJ40. pJ40 can restore Ced-3+ phenotype when microinjected into a *ced-3* mutant. pJ40 was subcloned by deleting a 2 kb *Bgl*II-*Apa*I fragment to generate pJ107. pJ107 was also able to rescue the Ced-3 phenotype when microinjected into a *ced-3* mutant. Deletion of 0.5 kb on the left side of pJ107 could be made by *Exo*III digestion (as in pJ107del28 and pJ107del34) without affecting Ced-3 activity; in fact, one transgenic line, *nEX17*, restores full Ced-3 activity. However, the *ced-3* rescuing ability was significantly reduced when 1 kb was deleted on the left side of pJ107 (as in pJ107del12 and pJ107del27), and the

ability was completely eliminated when a 1.8 kb *SalI-BglII* fragment was deleted on the right side of pJ107 (as in pJ55 and pJ56), suggesting that this *SalI* site is likely to be in the *ced-3* coding region. From these experiments, *ced-3* was localized to a DNA fragment of 7.5 kb. These results are summarized in Figure 2 and Table 4.

ced-3 Transcript

pJ107 was used to probe a Northern blot of N2 RNA and detected a band of 2.8 kb. Although this transcript is present in 12 *ced-3* mutant animals, subsequent analysis showed that all 12 *ced-3* mutant alleles contain mutations in the genomic DNA that codes for this mRNA (see below), thus establishing this RNA as a *ced-3* transcript.

The developmental expression pattern of *ced-3* was determined by hybridizing a Northern blot of RNA from animals of different stages (eggs, L1 through L4 larvae and young adult) with the *ced-3* cDNA subclone pJ118. Such analysis revealed that the *ced-3* transcript is most abundant during embryonic development, which is the period when most programmed cell deaths occur, but it was also detected during the L1 through L4 larval stages and is present in relatively high levels in young adults. This result suggests that *ced-3* is not only expressed in cells undergoing programmed cell death.

Since *ced-3* and *ced-4* are both required for programmed cell death in *C. elegans*, one of the genes might act as a regulator of transcription of the other gene. To examine if *ced-4* regulates the transcription of *ced-3*, RNA was prepared from eggs of *ced-4* mutants (*n1162, n1416, n1894*, and *n1920*), and a Northern blot was probed with the *ced-3* cDNA subclone pJ118. The presence of RNA in each lane was confirmed with an actin

I probe. Such an experiment showed that the level of *ced-3* transcript is normal in *ced-4* mutants. This indicates that *ced-4* is unlikely to be a transcriptional regulator of *ced-3*.

<u>Isolation of a *ced-3* cDNA</u>

To isolate cDNA of *ced-3*, pJ40 was used as a probe to screen a cDNA library of N2 (Kim and Horvitz, *Genes & Dev. 4*:357-371 (1990)). Seven cDNA clones were isolated. These cDNAs can be divided into two groups: one is 3.5 kb and the other 2.5 kb. One cDNA from each group was subcloned and analyzed further. pJ85 contains the 3.5 kb cDNA. Experiments showed that pJ85 contains a *ced-3* cDNA fused to an unrelated cDNA; on Northern blots of N2 RNA, the pJ85 insert hybridizes to two RNA transcripts, and on Southern blots of N2 DNA, pJ85 hybridizes to one more band than pJ40 (*ced-3* genomic DNA) does. pJ87 contains the 2.5 kb cDNA. On Northern blots, pJ87 hybridizes to a 2.8 kb RNA and on Southern blots, it hybridizes only to bands to which pJ40 hybridizes. Thus, pJ87 contains only *ced-3* cDNA.

To show that pJ87 does contain the *ced-3* cDNA, a frameshift mutation was made in the *Sal*I site of pJ40 corresponding to the *Sal*I site in the pJ87 cDNA. Constructs containing the frameshift mutation failed to rescue the Ced-3 phenotype when microinjected into *ced-3* mutant animals, suggesting that *ced-3* activity has been eliminated.

<u>*ced-3* Sequence</u>

The DNA sequence of pJ87 was determined (Figure 3). pJ87 contains an insert of 2.5 kb which has an open reading frame of 503 amino acids (Figure 3; SEQ ID NO:2). The 5' end of the cDNA contains 25 bp of poly-A/T sequence, which is probably an artifact of cloning

and is not present in the genomic sequence. The cDNA ends with a poly-A sequence, suggesting that it contains the complete 3′ end of the transcript. 1 kb of pJ87 insert is untranslated 3′ region and not all of it is essential for ced-3 expression, since genomic constructs with deletions of 380 bp of the 3′ end can still rescue *ced-3* mutants (pJ107 and its derivatives, see Figure 2).

To confirm the DNA sequence obtained from the *ced-3* cDNA and to study the structure of the *ced-3* gene, the genomic sequence of the *ced-3* gene in the plasmid pJ107 was determined (Figure 3; SEQ ID NO:1). Comparison of the *ced-3* genomic and cDNA sequences revealed that the *ced-3* gene has seven introns that range in size from 54 bp to 1195 bp (Figure 4A). The four largest introns, as well as sequences 5′ of the start codon, were found to contain repetitive elements (Figure 3). Five types of repetitive elements were found, some of which have been previously characterized in non-coding regions of other *C. elegans* genes, such as *fem-1* (Spence *et al.*, *Cell 60*:981-990 (1990)), *lin-12* (J. Yochem, personal communication), and *myoD* (Krause *et al.*, *Cell 63*:907-919 (1990)). Of these, repeat 1 was also found in *fem-1* and *myoD*, repeat 3 in *lin-12* and *fem-1*, repeat 4 in *lin-12*, and repeats 2 and 5 were novel repetitive elements.

A combination of primer extension and PCR amplification was used to determine the location and nature of the 5′ end of the *ced-3* transcript. Two primers (Pex1 and Pex2) were used for the primer extension reaction. The Pex1 reaction yielded two major bands, whereas the Pex2 reaction gave one band. The Pex2 band corresponded in size to the smaller band from the Pex1 reaction, and agreed in length with a possible transcript that is trans-spliced to a *C. elegans* splice leader (Bektesh, *Genes & Devel. 2*:1277-1283 (1988)) at a consensus splice acceptor at position 2166 of the

genomic sequence (Figure 3). The nature of the larger Pex1 band is unclear.

To confirm the existence of this trans-spliced message in wild-type worms, total *C. elegans* RNA was PCR amplified using the SL1-Log5 and SL2-Log5 primer pairs, followed by a reamplification using the SL1-Oligo10 and SL2-Oligo10 primer pairs. The SL1 reaction yielded a fragment of the predicted length. The identity of this fragment was confirmed by sequencing. Thus, at least some, if not most, of the *ced-3* transcript is trans-spliced to SL1. Based on this result, the start codon of the *ced-3* message was assigned to the methionine encoded at position 2232 of the genomic sequence (Figure 3).

The DNA sequences of 12 EMS-induced *ced-3* alleles were also determined (Figure 3 and Table 1). Nine of the 12 are missense mutations. Two of the 12 are nonsense mutations, which might prematurely terminate the translation of *ced-3*. These nonsense *ced-3* mutants confirmed that the *ced-3* gene is not essential for viability. One of the 12 mutations is an alteration of a conserved splicing acceptor G, and another has a change of a 70% conserved C at the splice site, which could also generate a stop codon even if the splicing is correct. Interestingly, these EMS-induced mutations are in either the N-terminal quarter or C-terminal half of the protein. In fact, 9 of the 12 mutations occur within the region of *ced-3* that encodes the last 100 amino acids of the protein. Mutations are notably absent from the middle part of the *ced-3* gene (Figure 4A).

<u>Ced-3 Protein Contains A Region Rich in Serines</u>

The Ced-3 protein is very hydrophilic and no significantly hydrophobic region can be found that might

be a trans-membrane domain (Figure 5). The Ced-3 protein is rich in serine. From amino acid 78 to amino acid 205 of the Ced-3 protein, 34 out of 127 amino acids are serine. Serine is often the target of serine/threonine protein kinases (Edelman, *Ann. Rev. Biochem.* *56*:567-613 (1987)). For example, protein kinase C can phosphorylate serines when they are flanked on their amino and carboxyl sides by basic residues (Edelman, 1987 *supra*). Four of the serines in the Ced-3 protein are flanked by arginines (Figures 6A-B). The same serine residues might also be the target of related Ser/Thr kinases.

To identify the functionally important regions of the Ced-3 protein, genomic DNAs containing the *ced-3* genes from two related nematode species, *C. briggsae* (SEQ ID NO:5) and *C. vulgaris* (SEQ ID NO:6) were cloned and sequenced. Sequence comparison of the three *ced-3* gene products showed that the non-serine-rich region of the proteins is highly conserved (Figure 9). In *C. briggsae* and *C. vulgaris*, many amino acids in the serine-rich region are dissimilar compared to the *C. elegans* Ced-3 protein. It seems that what is important in the serine-rich region is the overall serine-rich feature rather than the exact amino acid sequence.

This hypothesis is also supported by analysis of *ced-3* mutations in *C. elegans*: none of the 12 EMS-induced mutations is in the serine-rich region, suggesting that mutations in this region might not affect the function of the Ced-3 protein and thus, could not be isolated in the screen for *ced-3* mutants.

EXAMPLE 2
A COMMON MECHANISM OF CELL DEATH IN VERTEBRATES AND INVERTEBRATES

Results from previous studies reported in the scientific literature suggest that cell deaths in a variety of organisms, including vertebrates as well as invertebrates, share a common mechanism which involves the activation of genes. These studies are consistent with the hypothesis that genes similar to the *C. elegans* *ced-3* and *ced-4* genes may be involved in the cell deaths that occur in vertebrates, although certain observations have led some to distinguish vertebrate cell deaths from the programmed cell deaths observed in such invertebrates as nematodes and insects. Some vertebrate cell deaths share certain characteristics with the programmed cell deaths in *C. elegans* that are controlled by *ced-3* and *ced-4*. For example, up to 14% of the neurons in the chick dorsal root ganglia die immediately after their births, before any signs of differentiation (Carr and Simpson, *Dev. Brain Res. 2*:57-162 (1982)). Genes like *ced-3* and *ced-4* could well function in this class of vertebrate cell death.

Genetic mosaic analysis has suggested that *ced-3* and *ced-4* genes are expressed by cells that undergo programmed cell death, so that these genes may not act through cell-cell interactions (Yuan and Horvitz, *Dev. Biol. 138*:33-41 (1990)). Many cell deaths in vertebrates seem different in that they appear to be controlled by interactions with target tissues. For example, it is thought that a deprivation of target-derived growth factors is responsible for vertebrate neuronal cell deaths (Hamburger and Oppenheim, *Neurosci. Comment. 1*:39-55 (1982)); Thoenen *et al.*, in: *Selective Neuronal Death*, Wiley, New York, 1987, Vol. 126, pp. 82-

85). However, even this class of cell death could involve genes like *ced-3* and *ced-4*, since pathways of cell death involving similar genes and mechanisms might be triggered in a variety of ways. Supporting this idea are several *in vitro* and *in vivo* studies which show that the deaths of vertebrate as well as invertebrate cells can be prevented by inhibitors of RNA and protein synthesis, suggesting that activation of genes are required for these cell deaths (Martin *et al.*, *J. Cell Biol. 106*:829-844 (1988); Cohen and Duke, *J. Immunol. 132*:38-42 (1984); Oppenheim and Prevette, *Neurosci. Abstr. 14*:368 (1988); Stanisic *et al.*, *Invest. Urol. 16*:19-22 (1978); Oppenheim *et al.*, *Dev. Biol. 138*:104-113 (1990); Fahrbach and Truman, in: *Selective Neuronal Death, Ciba Foundation Symposium*, 1987, No. 126, pp. 65-81). It is possible that the genes induced in these dying vertebrate and invertebrate cells are cell death genes which are structurally related to the *C. elegans ced-3* or *ced-4* genes.

Also supporting the hypothesis that cell death in *C. elegans* is mechanistically similar to cell death in vertebrates is the observaiton that the protein product of the *C. elegans* gene *ced-9* is similar in sequence to the human protein Bcl-2. *ced-9* has been shown to prevent cells from undergoing programmed cell death during nematode development by antagonizing the activities of *ced-3* and *ced-4* (Hengartner, *et al.*, *Nature 356*:494-499 (1992)). The *bcl-2* gene has also been implicated in protecting cells against cell death. It seems likely that the genes and proteins with which *ced-9* and *bcl-2* interact are similar as well.

Table 1

Sites of Mutations in the *ced-3* Gene

Allele	Mutation	Nucleotide	Codon	Consequence
n1040	C to T	2310	27	L to F
n718	G to A	2487	65	G to R
n2433	G to A	5757	360	G to S
n1164	C to T	5940	403	Q to termination
n717	G to A	6297	-	Splice acceptor loss
n1949	C to T	6322	412	Q to termination
n1286	G to A	6342	428	W to termination
n1129	C to T	6434	449	A to V
n1165	C to T	6434	449	A to V
n2430	C to T	6485	466	A to V
n2426	G to A	6535	483	E to K
n1163	C to T	7020	486	S to F

Nucleotide and codon positions correspond to the numbering in Figure 3.

Table 2

***ced-3-lacZ* Fusions Which Prevent Programmed Cell Death**

Strain Name	Construct	Average # Extra Cells	Number of Animals
N2 (wild-type)	-	0.1	40
nEx 121	PBA	2.0	23
nEx 70	PBA	2.4	31
nEx 67	BGAFQ	2.1	18
nEx 66	BGAFQ	2.1	25

Table 3

Summary of Transformation Experiments Using Cosmids in the *ced-3* Region

Cosmid injected	No. of non-Unc transformants	Ced-3 phenotype	Strain name
C43C9; C14G10	1	-	MT4302
W07H6; C14G10	3	-	MT4299
		-	MT4300
		-	MT4301
C48D1; C14G10	2	+	MT4298
		+	MT4303

Animals injected were of genotype: *ced-1(e1735); unc-31(e929) ced-3(n717)*.

Table 4

The expression of *ced-3(+)* transformants

Genotype	DNA injected	Average No. cell deaths in L1 head	No. Animals scored
ced-1	-	23	20
ced-1; ced-3	-	0.3	10
ced-1; nIS1 unc-31 ced-3	C48D1; C14G10	16.4	20
ced-1; unc-31 ced-3; nIS1/+		14.5	20
ced-1; unc-31 ced-3; nEX2	C48D1; C14G10	13.2	10/14
		0	4/14
ced-1; unc-31 ced-3; nEX10	C48D1-28; C14G10	12	9/10
		0	1 of 10
ced-1; unc-31 ced-3; nEX9	C48D1-28; C14G10	12	10
ced-1; unc-31 ced-3; nEX11	C48D1-43 C14G10	16.7	10/13
		Abnormal cell deaths	3/13
ced-1; unc-31 ced-3; nEX13	pJ40; C14G10	13.75	4/4

Table 4 continued

ced-1; unc-31 ced-3; nEX17	pJ107de128, pJ107de134 C14G10	23	12/14
		0	2/14
ced-1; unc-31 ced-3; nEX18	pJ107de128, pJ107del134 C14G10	12.8	9/10
		0	1/10
ced-1; unc-31 ced-3; nEX19	pJ107de128, pJ107de134 G14G10	10.6	5/6
		0	1/6
ced-1; unc-31 ced-3; nEX16	pJ107del12, pJ107del27 C14G10	7.8	12/12

Alleles of the genes used are *ced-1(e1735)*, *unc-31(e928)*, and *ced-3(n717)*.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT
(A) NAME: Massachusetts Institute of Technology
(B) STREET: 77 Massachusetts Avenue
(C) CITY: Cambridge
(D) STATE OR PROVINCE: Massachusetts
(E) COUNTRY: U.S.A.
(F) POSTAL CODE: 02139

(ii) TITLE OF INVENTION: Inhibitors of Ced-3 and Related Proteins

(iii) NUMBER OF SEQUENCES: 14

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: diskette

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7653 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

AGATCTGAAA TAAGGTGATA AATTAATAAA TTAAGTGTAT TTCTGAGGAA ATTTGACTGT 60

TTTAGCACAA TTAATCTTGT TTCAGAAAAA AAGTCCAGTT TTCTAGATTT TTCCGTCTTA 120

TTGTCGAATT AATATCCCTA TTATCACTTT TTCATGCTCA TCCTCGAGCG GCACGTCCTC 180

AAAGAATTGT GAGAGCAAAC GCGCTCCCAT TGACCTCCAC ACTCAGCCGC CAAAACAAAC 240

GTTCGAACAT TCGTGTGTTG TGCTCCTTTT CCGTTATCTT GCAGTCATCT TTTGTCGTTT 300

TTTTCTTTGT TCTTTTTGTT GAACGTGTTG CTAAGCAATT ATTACATCAA TTGAAGAAAA 360

GGCTCGCCGA TTTATTGTTG CCAGAAAGAT TCTGAGATTC TCGAAGTCGA TTTTATAATA 420

TTTAACCTTG GTTTTTGCAT TGTTTCGTTT AAAAAAACCA CTGTTTATGT GAAAAACGAT 480

TAGTTTACTA ATAAAACTAC TTTTAAACCT TTACCTTTAC CTCACCGCTC CGTGTTCATG 540

GCTCATAGAT TTTCGATACT CAAATCCAAA AATAAATTTA CGAGGGCAAT TAATGTGAAA 600

CAAAAACAAT CCTAAGATTT CCACATGTTT GACCTCTCCG GCACCTTCTT CCTTAGCCCC 660

ACCACTCCAT CACCTCTTTG GCGGTGTTCT TCGAAACCCA CTTAGGAAAG CAGTGTGTAT 720

CTCATTTGGT ATGCTCTTTT CGATTTTATA GCTCTTTGTC GCAATTTCAA TGCTTTAAAC 780

AATCCAAATC GCATTATATT TGTGCATGGA GGCAAATGAC GGGGTTGGAA TCTTAGATGA 840

GATCAGGAGC TTTCAGGGTA AACGCCCGGT TCATTTTGTA CCACATTTCA TCATTTTCCT 900

GTCGTCCTTG GTATCCTCAA CTTGTCCCGG TTTTGTTTTC GGTACACTCT TCCGTGATGC 960

CACCTGTCTC CGTCTCAATT ATCGTTTAGA AATGTGAACT GTCCAGATGG GTGACTCATA 1020

TTGCTGCTGC TACAATCCAC TTTCTTTTCT CATCGGCAGT CTTACGAGCC CATCATAAAC 1080

TTTTTTTTCC GCGAAATTTG CAATAAACCG GCCAAAAACT TTCTCCAAAT TGTTACGCAA 1140

TATATACAAT CCATAAGAAT ATCTTCTCAA TGTTTATGAT TTCTTCGCAG CACTTTCTCT 1200

TCGTGTGCTA ACATCTTATT TTTATAATAT TTCCGCTAAA ATTCCGATTT TTGAGTATTA 1260

ATTTATCGTA AAATTATCAT AATAGCACCG AAAACTACTA AAAATGGTAA AAGCTCCTTT 1320

TAAATCGGCT CGACATTATC GTATTAAGGA ATCACAAAAT TCTGAGAATG CGTACTGCGC 1380

AACATATTTG ACGGCAAAAT ATCTCGTAGC GAAAACTACA GTAATTCTTT AAATGACTAC 1440

TGTAGCGCTT GTGTCGATTT ACGGGCTCAA TTTTTGAAAA TAATTTTTTT TTTCGAATTT 1500

TGATAACCCG TAAATCGTCA CAACGCTACA GTAGTCATTT AAAGGATTAC TGTAGTTCTA 1560

GCTACGAGAT ATTTTGCGCG CCAAATATGA CTGTAATACG CATTCTCTGA ATTTTGTGTT 1620

TCCGTAATAA TTTCACAAGA TTTTGGCATT CCACTTTAAA GGCGCACAGG ATTTATTCCA 1680

ATGGGTCTCG GCACGCAAAA AGTTTGATAG ACTTTTAAAT TCTCCTTGCA TTTTTAATTC 1740

AATTACTAAA ATTTTCGTGA ATTTTTCTGT TAAAATTTTT AAAATCAGTT TTCTAATATT 1800

TTCCAGGCTG ACAAACAGAA ACAAAAACAC AACAAACATT TTAAAAATCA GTTTTCAAAT 1860

TAAAAATAAC GATTTCTCAT TGAAAATTGT GTTTTATGTT TGCGAAAATA AAAGAGAACT 1920

GATTCAAAAC AATTTTAACA AAAAAAAACC CCAAAATTCG CCAGAAATCA AGATAAAAAA 1980

TTCAAGAGGG TCAAAATTTT CCGATTTTAC TGACTTTCAC CTTTTTTTTC GTAGTTCAGT 2040

GCAGTTGTTG GAGTTTTTGA CGAAAACTAG GAAAAAAATC GATAAAAATT ACTCAAATCG 2100

AGCTGAATTT TGAGGACAAT GTTTAAAAAA AAACACTATT TTTCCAATAA TTTCACTCAT 2160

TTTCAGACTA AATCGAAAAT CAAATCGTAC TCTGACTACG GGTCAGTAGA GAGGTCAACC 2220

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TCTCTAGTCA TCTAAAAGTC GATGAAATTC TCGAAGTTCT CATCGCAAAA CAAGTGTTGA 2340

ATAGTGATAA TGGAGATATG ATTAATGTGA GTTTTTAATC GAATAATAAT TTTAAAAAAA 2400

AATTGATAAT ATAAAGAATA TTTTTGCAGT CATGTGGAAC GGTTCGCGAG AAGAGACGGG 2460

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TTCGCTCTAC GGGACACGAA GGACTTGCTG AAGTTCTTGA ACCTCTCGCC AGATCGTAGG 2580
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GCAACCCACC GGCACGGTTT TTTCCTCCGA AAATCGGAAA TTATGCACTT TCCCAAATAT 2700
TTGAAGTGAA ATATATTTTA TTTACTGAAA GCTCGAGTGA TTATTTATTT TTTAACACTA 2760
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ACACACATCT CCTTCAAATA TCCCTTTTTC CAGTGTTGAC TCGAATGCTG TCGAATTCGA 2880
GTGTCCAATG TCACCGGCAA GCCATCGTCG GAGCCGCGCA TTGAGCCCCG CCGGCTACAC 2940
TTCACCGACC CGAGTTCACC GTGACAGCGT CTCTTCAGTG TCATCATTCA CTTCTTATCA 3000
GGATATCTAC TCAAGAGCAA GATCTCGTTC TCGATCGCGT GCACTTCATT CATCGGATCG 3060
ACACAATTAT TCATCTCCTC CAGTCAACGC ATTTCCCAGC CAACCTTGTA TGTTGATGCG 3120
AACACTAAAT TCTGAGAATG CGCATTACTC AACATATTTG ACGCGCAAAT ATCTCGTAGC 3180
GAAAAATACA GTAACCCTTT AAATGACTAT TGTAGTGTCG ATTTACGGGC TCGATTTTCG 3240
AAACGAATAT ATGCTCGAAT TGTGACAACG AATTTTAATT TGTCATTTTT GTGTTTTCTT 3300
TTGATATTTT TGATCAATTA ATAAATTATT TCCGTAAACA GACACCAGCG CTACAGTACT 3360
CTTTTAAAGA GTTACAGTAG TTTTCGCTTC AAGATATTTT GAAAAGAATT TTAAACATTT 3420
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TTTTTTTCAT TCAAGATATG CTTATTAACA CATATAATTA TCATTAATGT GAATTTCTTG 3540
TAGAAATTTT GGGCTTTTCG TTCTAGTATG CTCTACTTTT GAAATTGCTC AACGAAAAAA 3600
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GAGCAACTTA AAAAGTAGAG AATATTAGAG CGAAAACCAA AATTTCTTCA AGATATTACC 4020
TTTATTGATA ATTATAGATG TTAATAAGCA TATCTTGAAT GAAAGTCAGC AAAAATATGT 4080
GCGAAACACC TGAAAAAAAT CAAAAATTCT GCGAAAATTG AAAAAATGCA TTAAAATACA 4140
TTTTTGCATT TTTCTACATC ACATGAATGT AGAAAATTAA AAGGGAAATC AAAATTTCTA 4200
GAGGATATAA TTGAATGAAA CATTGCGAAA TTAAAATGTG CGAAACGTCA AAAAAGAGGA 4260

AATTTGGGTA TCAAAATCGA TCCTAAAACC AACACATTTC AGCATCCGCC AACTCTTCAT 4320
TCACCGGATG CTCTTCTCTC GGATACAGTT CAAGTCGTAA TCGCTCATTC AGCAAAGCTT 4380
CTGGACCAAC TCAATACATA TTCCATGAAG AGGATATGAA CTTTGTCGAT GCACCAACCA 4440
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ACAAGGACAA TCTTACCAAT TTGTTCAGAT GCATGGGCTA TACGGTTATT TGCAAGGACA 4620
ATCTGACGGG AAGGGTACGG CGAAATTATA TTACCCAAAC GCGAAATTTG CCATTTTGCG 4680
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ACCAAAAATT TGTCGTGTCG AGACCAGGTA CCGTAGTTTT TGTCGCAAAA ATTGCACCAT 5340
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AAATCAATTT TCTGCAAAAT ACCAAAAAGA AACCCGAAAA AATTTCCCAG CCTTGTTCCT 5520
AATGTAAACT GATATTTAAT TTCCAGGGAA TGCTCCTGAC AATTCGAGAC TTTGCCAAAC 5580
ACGAATCACA CGGAGATTCT GCGATACTCG TGATTCTATC ACACGGAGAA GAGAATGTGA 5640
TTATTGGAGT TGATGATATA CCGATTAGTA CACACGAGAT ATATGATCTT CTCAACGCGG 5700
CAAATGCTCC CCGTCTGGCG AATAAGCCGA AAATCGTTTT TGTGCAGGCT TGTCGAGGCG 5760
GTTCGTTTTT TATTTTAATT TTAATATAAA TATTTTAAAT AAATTCATTT TCAGAACGTC 5820
GTGACAATGG ATTCCCAGTC TTGGATTCTG TCGACGGAGT TCCTGCATTT CTTCGTCGTG 5880
GATGGGACAA TCGAGACGGG CCATTGTTCA ATTTTCTTGG ATGTGTGCGG CCGCAAGTTC 5940
AGGTTGCAAT TTAATTTCTT GAATGAGAAT ATTCCTTCAA AAAATCTAAA ATAGATTTTT 6000

ATTCCAGAAA GTCCCGATCG AAAAATTGCG ATATAATTAC GAAATTTGTG ATAAAATGAC 6060
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GGAGCGAAAG CCCCGTCCTG TAAACATTTT TAAATGATAA TTAATAAATT TTTGCAGCAA 6300
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GCATTTAAGC CAAAATTGTT AACTCATTTA AAAATTAATT CAAAGTTGTC CACGAGTATT 6900
ACACGGTTGG CGCGCGGCAA GTTTGCAAAA CGACGCTCCG CCTCTTTTTC TGTGCGGCTT 6960
GAAAACAAGG GATCGGTTTA GATTTTTCCC CAAAATTTAA ATTAAATTTC AGATGACATC 7020
CCGCCTGCTC AAAAAGTTCT ACTTTTGGCC GGAAGCACGA AACTCTGCCG TCTAAAATTC 7080
ACTCGTGATT CATTGCCCAA TTGATAATTG TCTGTATCTT CTCCCCCAGT TCTCTTTCGC 7140
CCAATTAGTT TAAAACCATG TGTATATTGT TATCCTATAC TCATTTCACT TTATCATTCT 7200
ATCATTTCTC TTCCCATTTT CACACATTTC CATTTCTCTA CGATAATCTA AAATTATGAC 7260
GTTTGTGTCT CGAACGCATA ATAATTTTAA TAACTCGTTT TGAATTTGAT TAGTTGTTGT 7320
GCCCAGTATA TATGTATGTA CTATGCTTCT ATCAACAAAA TAGTTTCATA GATCATCACC 7380
CCAACCCCAC CAACCTACCG TACCATATTC ATTTTTGCCG GGAATCAATT TCGATTAATT 7440
TTAACCTATT TTTTCGCCAC AAAAAATCTA ATATTTGAAT TAACGAATAG CATTCCCATC 7500
TCTCCCGTGC CGGAATGCCT CCCGGCCTTT TAAAGTTCGG AACATTTGGC AATTATGTAT 7560
AAATTTGTAG GTCCCCCCCA TCATTTCCCG CCCATCATCT CAAATTGCAT TCTTTTTTCG 7620
CCGTGATATC CCGATTCTGG TCAGCAAAGA TCT 7653

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 503 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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Met Met Arg Gln Asp Arg Arg Ser Leu Leu Glu Arg Asn Ile Met Met
1               5                   10                  15

Phe Ser Ser His Leu Lys Val Asp Glu Ile Leu Glu Val Leu Ile Ala
            20                  25                  30

Lys Gln Val Leu Asn Ser Asp Asn Gly Asp Met Ile Asn Ser Cys Gly
        35                  40                  45

Thr Val Arg Glu Lys Arg Arg Glu Ile Val Lys Ala Val Gln Arg Arg
    50                  55                  60

Gly Asp Val Ala Phe Asp Ala Phe Tyr Asp Ala Leu Arg Ser Thr Gly
65                  70                  75                  80

His Glu Gly Leu Ala Glu Val Leu Glu Pro Leu Ala Arg Ser Val Asp
                85                  90                  95

Ser Asn Ala Val Glu Phe Glu Cys Pro Met Ser Pro Ala Ser His Arg
            100                 105                 110

Arg Ser Arg Ala Leu Ser Pro Ala Gly Tyr Thr Ser Pro Thr Arg Val
        115                 120                 125

His Arg Asp Ser Val Ser Ser Val Ser Ser Phe Thr Ser Tyr Gln Asp
    130                 135                 140

Ile Tyr Ser Arg Ala Arg Ser Arg Ser Arg Ser Arg Ala Leu His Ser
145                 150                 155                 160

Ser Asp Arg His Asn Tyr Ser Ser Pro Pro Val Asn Ala Phe Pro Ser
                165                 170                 175

Gln Pro Ser Ser Ala Asn Ser Ser Phe Thr Gly Cys Ser Ser Leu Gly
            180                 185                 190

Tyr Ser Ser Ser Arg Asn Arg Ser Phe Ser Lys Ala Ser Gly Pro Thr
        195                 200                 205

Gln Tyr Ile Phe His Glu Glu Asp Met Asn Phe Val Asp Ala Pro Thr
    210                 215                 220

Ile Ser Arg Val Phe Asp Glu Lys Thr Met Tyr Arg Asn Phe Ser Ser
225                 230                 235                 240

Pro Arg Gly Met Cys Leu Ile Ile Asn Asn Glu His Phe Glu Gln Met
                245                 250                 255
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Pro Thr Arg Asn Gly Thr Lys Ala Asp Lys Asp Asn Leu Thr Asn Leu
            260                 265                 270

Phe Arg Cys Met Gly Tyr Thr Val Ile Cys Lys Asp Asn Leu Thr Gly
        275                 280                 285

Arg Gly Met Leu Leu Thr Ile Arg Asp Phe Ala Lys His Glu Ser His
    290                 295                 300

Gly Asp Ser Ala Ile Leu Val Ile Leu Ser His Gly Glu Glu Asn Val
305                 310                 315                 320

Ile Ile Gly Val Asp Asp Ile Pro Ile Ser Thr His Glu Ile Tyr Asp
                325                 330                 335

Leu Leu Asn Ala Ala Asn Ala Pro Arg Leu Ala Asn Lys Pro Lys Ile
            340                 345                 350

Val Phe Val Gln Ala Cys Arg Gly Glu Arg Arg Asp Asn Gly Phe Pro
        355                 360                 365

Val Leu Asp Ser Val Asp Gly Val Pro Ala Phe Leu Arg Arg Gly Trp
    370                 375                 380

Asp Asn Arg Asp Gly Pro Leu Phe Asn Phe Leu Gly Cys Val Arg Pro
385                 390                 395                 400

Gln Val Gln Gln Val Trp Arg Lys Lys Pro Ser Gln Ala Asp Ile Leu
                405                 410                 415

Ile Arg Tyr Ala Thr Thr Ala Gln Tyr Val Ser Trp Arg Asn Ser Ala
            420                 425                 430

Arg Gly Ser Trp Phe Ile Gln Ala Val Cys Glu Val Phe Ser Thr His
        435                 440                 445

Ala Lys Asp Met Asp Val Val Glu Leu Leu Thr Glu Val Asn Lys Lys
    450                 455                 460

Val Ala Cys Gly Phe Gln Thr Ser Gln Gly Ser Asn Ile Leu Lys Gln
465                 470                 475                 480

Met Pro Glu Met Thr Ser Arg Leu Leu Lys Lys Phe Tyr Phe Trp Pro
                485                 490                 495

Glu Ala Arg Asn Ser Ala Val
            500
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 1373 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

```
    (ix) FEATURE:
         (A) NAME/KEY: CDS
         (B) LOCATION: 18..1232
         (D) OTHER INFORMATION: /product= "human interleukin-1β
              convertase"

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

AAAAGGAGAG AAAAGCC ATG GCC GAC AAG GTC CTG AAG GAG AAG AGA AAG        50
                   Met Ala Asp Lys Val Leu Lys Glu Lys Arg Lys
                    1               5                  10

CTG TTT ATC CGT TCC ATG GGT GAA GGT ACA ATA AAT GGC TTA CTG GAT       98
Leu Phe Ile Arg Ser Met Gly Glu Gly Thr Ile Asn Gly Leu Leu Asp
             15                  20                  25

GAA TTA TTA CAG ACA AGG GTG CTG AAC AAG GAA GAG ATG GAG AAA GTA      146
Glu Leu Leu Gln Thr Arg Val Leu Asn Lys Glu Glu Met Glu Lys Val
         30                  35                  40

AAA CGT GAA AAT GCT ACA GTT ATG GAT AAG ACC CGA GCT TTG ATT GAC      194
Lys Arg Glu Asn Ala Thr Val Met Asp Lys Thr Arg Ala Leu Ile Asp
     45                  50                  55

TCC GTT ATT CCG AAA GGG GCA CAG GCA TGC CAA ATT TGC ATC ACA TAC      242
Ser Val Ile Pro Lys Gly Ala Gln Ala Cys Gln Ile Cys Ile Thr Tyr
 60                  65                  70                  75

ATT TGT GAA GAA GAC AGT TAC CTG GCA GGG ACG CTG GGA CTC TCA GCA      290
Ile Cys Glu Glu Asp Ser Tyr Leu Ala Gly Thr Leu Gly Leu Ser Ala
                 80                  85                  90

GAT CAA ACA TCT GGA AAT TAC CTT AAT ATG CAA GAC TCT CAA GGA GTA      338
Asp Gln Thr Ser Gly Asn Tyr Leu Asn Met Gln Asp Ser Gln Gly Val
             95                 100                 105

CTT TCT TCC TTT CCA GCT CCT CAG GCA GTG CAG GAC AAC CCA GCT ATG      386
Leu Ser Ser Phe Pro Ala Pro Gln Ala Val Gln Asp Asn Pro Ala Met
        110                 115                 120

CCC ACA TCC TCA GGC TCA GAA GGG AAT GTC AAG CTT TGC TCC CTA GAA      434
Pro Thr Ser Ser Gly Ser Glu Gly Asn Val Lys Leu Cys Ser Leu Glu
    125                 130                 135

GAA GCT CAA AGG ATA TGG AAA CAA AAG TCG GCA GAG ATT TAT CCA ATA      482
Glu Ala Gln Arg Ile Trp Lys Gln Lys Ser Ala Glu Ile Tyr Pro Ile
140                 145                 150                 155

ATG GAC AAG TCA AGC CGC ACA CGT CTT GCT CTC ATT ATC TGC AAT GAA      530
Met Asp Lys Ser Ser Arg Thr Arg Leu Ala Leu Ile Ile Cys Asn Glu
                160                 165                 170

GAA TTT GAC AGT ATT CCT AGA AGA ACT GGA GCT GAG GTT GAC ATC ACA      578
Glu Phe Asp Ser Ile Pro Arg Arg Thr Gly Ala Glu Val Asp Ile Thr
            175                 180                 185
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GGC ATG ACA ATG CTG CTA CAA AAT CTG GGG TAC AGC GTA GAT GTG AAA       626
Gly Met Thr Met Leu Leu Gln Asn Leu Gly Tyr Ser Val Asp Val Lys
        190                 195                 200

AAA AAT CTC ACT GCT TCG GAC ATG ACT ACA GAG CTG GAG GCA TTT GCA       674
Lys Asn Leu Thr Ala Ser Asp Met Thr Thr Glu Leu Glu Ala Phe Ala
    205                 210                 215

CAC CGC CCA GAG CAC AAG ACC TCT GAC AGC ACG TTC CTG GTG TTC ATG       722
His Arg Pro Glu His Lys Thr Ser Asp Ser Thr Phe Leu Val Phe Met
220                 225                 230                 235

TCT CAT GGT ATT CGG GAA GGC ATT TGT GGG AAG AAA CAC TCT GAG CAA       770
Ser His Gly Ile Arg Glu Gly Ile Cys Gly Lys Lys His Ser Glu Gln
            240                 245                 250

GTC CCA GAT ATA CTA CAA CTC AAT GCA ATC TTT AAC ATG TTG AAT ACC       818
Val Pro Asp Ile Leu Gln Leu Asn Ala Ile Phe Asn Met Leu Asn Thr
        255                 260                 265

AAG AAC TGC CCA AGT TTG AAG GAC AAA CCG AAG GTG ATC ATC ATC CAG       866
Lys Asn Cys Pro Ser Leu Lys Asp Lys Pro Lys Val Ile Ile Ile Gln
        270                 275                 280

GCC TGC CGT GGT GAC AGC CCT GGT GTG GTG TGG TTT AAA GAT TCA GTA       914
Ala Cys Arg Gly Asp Ser Pro Gly Val Val Trp Phe Lys Asp Ser Val
    285                 290                 295

GGA GTT TCT GGA AAC CTA TCT TTA CCA ACT ACA GAA GAG TTT GAG GAT       962
Gly Val Ser Gly Asn Leu Ser Leu Pro Thr Thr Glu Glu Phe Glu Asp
300                 305                 310                 315

GAT GCT ATT AAG AAA GCC CAC ATA GAG AAG GAT TTT ATC GCT TTC TGC      1010
Asp Ala Ile Lys Lys Ala His Ile Glu Lys Asp Phe Ile Ala Phe Cys
            320                 325                 330

TCT TCC ACA CCA GAT AAT GTT TCT TGG AGA CAT CCC ACA ATG GGC TCT      1058
Ser Ser Thr Pro Asp Asn Val Ser Trp Arg His Pro Thr Met Gly Ser
            335                 340                 345

GTT TTT ATT GGA AGA CTC ATT GAA CAT ATG CAA GAA TAT GCC TGT TCC      1106
Val Phe Ile Gly Arg Leu Ile Glu His Met Gln Glu Tyr Ala Cys Ser
        350                 355                 360

TGT GAT GTG GAG GAA ATT TTC CGC AAG GTT CGA TTT TCA TTT GAG CAG      1154
Cys Asp Val Glu Glu Ile Phe Arg Lys Val Arg Phe Ser Phe Glu Gln
    365                 370                 375

CCA GAT GGT AGA GCG CAG ATG CCC ACC ACT GAA AGA GTG ACT TTG ACA      1202
Pro Asp Gly Arg Ala Gln Met Pro Thr Thr Glu Arg Val Thr Leu Thr
380                 385                 390                 395

AGA TGT TTC TAC CTC TTC CCA GGA CAT TAAAATAAGG AAACTGTATG            1249
Arg Cys Phe Tyr Leu Phe Pro Gly His
            400                 405

AATGTCTGCG GGCAGGAAGT GAAGAGATCG TTCTGTAAAA GGTTTTTGGA ATTATGTCTG    1309
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CTGAATAATA AACTTTTTTT GAAATAATAA ATCTGGTAGA AAAATGAAAA AAAAAAAAAA 1369

AAAA 1373

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 404 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Ala Asp Lys Val Leu Lys Glu Lys Arg Lys Leu Phe Ile Arg Ser
1 5 10 15

Met Gly Glu Gly Thr Ile Asn Gly Leu Leu Asp Glu Leu Leu Gln Thr
20 25 30

Arg Val Leu Asn Lys Glu Glu Met Glu Lys Val Lys Arg Glu Asn Ala
35 40 45

Thr Val Met Asp Lys Thr Arg Ala Leu Ile Asp Ser Val Ile Pro Lys
50 55 60

Gly Ala Gln Ala Cys Gln Ile Cys Ile Thr Tyr Ile Cys Glu Glu Asp
65 70 75 80

Ser Tyr Leu Ala Gly Thr Leu Gly Leu Ser Ala Asp Gln Thr Ser Gly
85 90 95

Asn Tyr Leu Asn Met Gln Asp Ser Gln Gly Val Leu Ser Ser Phe Pro
100 105 110

Ala Pro Gln Ala Val Gln Asp Asn Pro Ala Met Pro Thr Ser Ser Gly
115 120 125

Ser Glu Gly Asn Val Lys Leu Cys Ser Leu Glu Glu Ala Gln Arg Ile
130 135 140

Trp Lys Gln Lys Ser Ala Glu Ile Tyr Pro Ile Met Asp Lys Ser Ser
145 150 155 160

Arg Thr Arg Leu Ala Leu Ile Ile Cys Asn Glu Glu Phe Asp Ser Ile
165 170 175

Pro Arg Arg Thr Gly Ala Glu Val Asp Ile Thr Gly Met Thr Met Leu
180 185 190

Leu Gln Asn Leu Gly Tyr Ser Val Asp Val Lys Lys Asn Leu Thr Ala
195 200 205

Ser Asp Met Thr Thr Glu Leu Glu Ala Phe Ala His Arg Pro Glu His
210 215 220

Lys Thr Ser Asp Ser Thr Phe Leu Val Phe Met Ser His Gly Ile Arg
225 230 235 240

Glu Gly Ile Cys Gly Lys Lys His Ser Glu Gln Val Pro Asp Ile Leu
245 250 255

Gln Leu Asn Ala Ile Phe Asn Met Leu Asn Thr Lys Asn Cys Pro Ser
260 265 270

Leu Lys Asp Lys Pro Lys Val Ile Ile Ile Gln Ala Cys Arg Gly Asp
275 280 285

Ser Pro Gly Val Val Trp Phe Lys Asp Ser Val Gly Val Ser Gly Asn
290 295 300

Leu Ser Leu Pro Thr Thr Glu Glu Phe Glu Asp Asp Ala Ile Lys Lys
305 310 315 320

Ala His Ile Glu Lys Asp Phe Ile Ala Phe Cys Ser Ser Thr Pro Asp
325 330 335

Asn Val Ser Trp Arg His Pro Thr Met Gly Ser Val Phe Ile Gly Arg
340 345 350

Leu Ile Glu His Met Gln Glu Tyr Ala Cys Ser Cys Asp Val Glu Glu
355 360 365

Ile Phe Arg Lys Val Arg Phe Ser Phe Glu Gln Pro Asp Gly Arg Ala
370 375 380

Gln Met Pro Thr Thr Glu Arg Val Thr Leu Thr Arg Cys Phe Tyr Leu
385 390 395 400

Phe Pro Gly His

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 505 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:
(A) NAME/KEY: unsure
(B) LOCATION: at every Xaa

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Met Met Arg Gln Asp Arg Trp Leu Leu Glu Arg Asn Ile Leu Glu Phe
1 5 10 15

Ser Ser Lys Leu Gln Ala Asp Leu Ile Leu Asp Val Leu Ile Ala Lys
20 25 30

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Gln Val Leu Asn Ser Asp Asn Gly Asp Val Ile Asn Ser Cys Arg Thr
        35                  40                  45

Glu Arg Asp Asn Glu Lys Glu Ile Val Lys Ala Val Gln Arg Arg Gly
    50                  55                  60

Asp Glu Ala Phe Asp Ala Phe Tyr Asp Ala Leu Arg Asp Thr Gly His
65                  70                  75                  80

Asn Asp Leu Ala Asp Val Leu Met Pro Leu Ser Arg Pro Xaa Xaa Xaa
                85                  90                  95

Asn Pro Val Pro Met Glu Cys Pro Met Ser Pro Ser Ser His Arg Arg
            100                 105                 110

Ser Arg Ala Leu Ser Pro Pro Xaa Tyr Ala Ser Pro Thr Arg Val His
        115                 120                 125

Arg Asp Ser Ile Ser Ser Val Ser Ser Phe Thr Ser Thr Tyr Gln Asp
    130                 135                 140

Val Tyr Ser Arg Ala Arg Ser Ser Ser Arg Ser Ser Arg Pro Leu Gln
145                 150                 155                 160

Ser Ser Asp Arg His Asn Tyr Met Ser Ala Ala Thr Ser Phe Pro Ser
                165                 170                 175

Gln Pro Xaa Ser Ala Asn Ser Ser Phe Thr Gly Cys Ala Ser Leu Gly
            180                 185                 190

Tyr Ser Ser Ser Arg Asn Arg Ser Phe Ser Lys Thr Ser Ala Gln Ser
        195                 200                 205

Gln Tyr Ile Phe His Glu Glu Asp Met Asn Tyr Val Asp Ala Pro Thr
    210                 215                 220

Ile His Arg Val Phe Asp Glu Lys Thr Met Tyr Arg Asn Phe Ser Ser
225                 230                 235                 240

Pro Arg Gly Leu Cys Leu Ile Ile Asn Asn Glu His Phe Glu Gln Met
                245                 250                 255

Pro Thr Arg Asn Gly Thr Lys Ala Asp Lys Asp Asn Leu Thr Asn Ile
            260                 265                 270

Phe Arg Cys Met Gly Tyr Thr Val Ile Cys Lys Asp Asn Leu Thr Gly
        275                 280                 285

Arg Glu Met Leu Ser Thr Ile Arg Ser Phe Gly Arg Asn Asp Met His
    290                 295                 300

Gly Asp Ser Ala Ile Leu Val Ile Leu Ser His Gly Glu Xaa Asn Val
305                 310                 315                 320

Ile Ile Gly Val Asp Asp Val Ser Val Asn Val His Glu Ile Tyr Asp
                325                 330                 335

Leu Leu Asn Ala Ala Asn Ala Pro Arg Leu Ala Asn Lys Pro Lys Leu
```

```
                340                     345                     350

Val Phe Val Gln Ala Cys Arg Gly Glu Arg Arg Asp Asn Gly Phe Pro
        355                     360                     365

Val Leu Asp Ser Val Asp Gly Val Pro Ser Leu Ile Arg Arg Gly Trp
    370                     375                     380

Asp Asn Arg Asp Gly Pro Leu Phe Asn Phe Leu Gly Cys Val Arg Pro
385                     390                     395                 400

Gln Val Gln Gln Val Trp Arg Lys Lys Pro Ser Gln Ala Asp Met Leu
                405                     410                     415

Ile Ala Tyr Ala Thr Thr Ala Gln Tyr Val Ser Trp Arg Asn Ser Ala
            420                     425                     430

Arg Gly Ser Trp Phe Ile Gln Ala Val Cys Glu Val Phe Ser Leu His
        435                     440                     445

Ala Lys Asp Met Asp Val Val Glu Leu Leu Thr Glu Val Asn Lys Lys
    450                     455                     460

Val Ala Cys Gly Phe Gln Thr Ser Gln Gly Ser Asn Ile Leu Lys Gln
465                     470                     475                 480

Met Pro Glu Leu Thr Ser Arg Leu Leu Lys Lys Phe Tyr Phe Trp Pro
                485                     490                     495

Glu Asp Arg Gly Arg Asn Ser Ala Val
            500                     505
```

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 480 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:
 (A) NAME/KEY: unsure
 (B) LOCATION: at every Xaa

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
Thr Val Ser Ile Ser Leu Ile Ile Ala Arg Gln Val Leu Asn Ser Asp
1               5                   10                  15

Asn Xaa Xaa Met Ile Asn Ser Cys Gly Xaa Xaa Xaa Xaa Xaa Xaa Xaa
            20                  25                  30

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
        35                  40                  45
```

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
50 55 60

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
65 70 75 80

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
85 90 95

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
100 105 110

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Ser Thr Ser
115 120 125

Arg Ser Ser Arg Pro Leu His Thr Ser Asp Arg His Asn Tyr Val Ser
130 135 140

Pro Ser Asn Ser Phe Gln Ser Gln Pro Ala Ser Ala Asn Ser Ser Phe
145 150 155 160

Thr Gly Ser Ser Ser Leu Gly Tyr Ser Ser Ser Arg Thr Arg Ser Tyr
165 170 175

Ser Lys Ala Ser Ala His Ser Gln Tyr Ile Phe His Glu Glu Asp Met
180 185 190

Asn Tyr Val Asp Ala Pro Thr Ile His Arg Val Phe Asp Glu Lys Thr
195 200 205

Met Tyr Arg Asn Phe Ser Thr Pro Arg Gly Leu Cys Leu Ile Ile Asn
210 215 220

Asn Glu His Phe Glu Gln Met Pro Thr Arg Asn Gly Thr Lys Pro Asp
225 230 235 240

Lys Asp Asn Ile Ser Asn Leu Phe Arg Cys Met Gly Tyr Ile Val His
245 250 255

Cys Lys Asp Asn Leu Thr Gly Arg Xaa Met Met Leu Thr Ile Arg Asp
260 265 270

Phe Ala Lys Asn Glu Thr His Gly Asp Ser Ala Ile Leu Val Ile Xaa
275 280 285

Ser His Gly Glu Glu Asn Val Ile Ile Gly Val Asp Asp Val Ser Val
290 295 300

Asn Val His Glu Ile Tyr Xaa Leu Leu Asn Ala Ala Asn Ala Pro Arg
305 310 315 320

Leu Ala Asn Lys Pro Lys Leu Val Phe Val Gln Ala Cys Arg Gly Glu
325 330 335

Arg Arg Asp Val Gly Phe Pro Val Leu Asp Ser Val Asp Gly Val Pro
340 345 350

Ala Leu Ile Arg Arg Gly Trp Asp Lys Gly Asp Gly Pro Xaa Xaa Asn
355 360 365

Phe Leu Gly Cys Val Arg Pro Gln Ala Gln Gln Val Trp Arg Lys Lys
370 375 380

Pro Ser Gln Ala Asp Ile Leu Ile Ala Tyr Ala Thr Thr Ala Gln Tyr
385 390 395 400

Val Ser Trp Arg Asn Ser Ala Arg Gly Ser Trp Phe Ile Gln Ala Val
405 410 415

Cys Glu Val Phe Ser Leu His Ala Lys Asp Met Asp Val Val Glu Leu
420 425 430

Leu Thr Glu Val Asn Lys Lys Val Ala Cys Gly Phe Gln Thr Ser Gln
435 440 445

Gly Ala Asn Ile Leu Lys Gln Met Pro Xaa Leu Thr Ser Arg Leu Leu
450 455 460

Lys Lys Phe Tyr Phe Trp Pro Glu Asp Arg Asn Arg Ser Ser Ala Val
465 470 475 480

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

TCATCGACTT TTAGATGACT AGAGAACATC 30

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GTTGCACTGC TTTCACGATC TCCCGTCTCT 30

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GTTTAATTAC CCAAGTTTGA G 21

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

GGTTTTAACC AGTTACTCAA G 21

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

CCGGTGACAT TGGACACTC 19

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

ACTATTCAAC ACTTG 15

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 171 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

```
Met Leu Thr Val Gln Val Tyr Arg Thr Ser Gln Lys Cys Ser Ser Ser
1               5                   10                  15
```

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Lys His Val Val Glu Val Leu Leu Asp Pro Leu Gly Thr Ser Phe Cys
            20                  25                  30

Ser Leu Leu Pro Pro Pro Leu Leu Leu Tyr Glu Thr Asp Arg Gly Val
        35                  40                  45

Asp Gln Gln Asp Gly Lys Asn His Thr Gln Ser Pro Gly Cys Glu Glu
    50                  55                  60

Ser Asp Ala Gly Lys Glu Glu Leu Met Lys Met Arg Leu Pro Thr Arg
65                  70                  75                  80

Ser Asp Met Ile Cys Gly Tyr Ala Cys Leu Lys Gly Asn Ala Ala Met
                85                  90                  95

Arg Asn Thr Lys Arg Gly Ser Trp Tyr Ile Glu Ala Leu Thr Gln Val
            100                 105                 110

Phe Ser Glu Arg Ala Cys Asp Met His Val Ala Asp Met Leu Val Lys
        115                 120                 125

Val Asn Ala Leu Ile Lys Glu Arg Glu Gly Tyr Ala Pro Gly Thr Glu
    130                 135                 140

Phe His Arg Cys Lys Glu Met Ser Glu Tyr Cys Ser Thr Leu Cys Gln
145                 150                 155                 160

Gln Leu Tyr Leu Phe Pro Gly Tyr Pro Pro Thr
                165                 170
```

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 402 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

```
Met Ala Asp Lys Ile Leu Arg Ala Lys Arg Lys Gln Phe Ile Asn Ser
1               5                   10                  15

Val Ser Ile Gly Thr Ile Asn Gly Leu Leu Asp Glu Leu Leu Glu Lys
            20                  25                  30

Arg Val Leu Asn Gln Glu Glu Met Asp Lys Ile Lys Leu Ala Asn Ile
        35                  40                  45

Thr Ala Met Asp Lys Ala Arg Asp Leu Cys Asp His Val Ser Lys Lys
    50                  55                  60

Gly Pro Gln Ala Ser Gln Ile Phe Ile Thr Tyr Ile Cys Asn Glu Asp
65                  70                  75                  80
```

Cys Tyr Leu Ala Gly Ile Leu Glu Leu Gln Ser Ala Pro Ser Ala Glu
85 90 95

Thr Phe Val Ala Thr Glu Asp Ser Lys Gly Gly His Pro Ser Ser Ser
100 105 110

Glu Thr Lys Glu Glu Gln Asn Lys Glu Asp Gly Thr Phe Pro Gly Leu
115 120 125

Thr Gly Thr Leu Lys Phe Cys Pro Leu Glu Lys Ala Gln Lys Leu Trp
130 135 140

Lys Glu Asn Pro Ser Glu Ile Tyr Pro Ile Met Asn Thr Thr Thr Arg
145 150 155 160

Thr Arg Leu Ala Leu Ile Ile Cys Asn Thr Glu Phe Gln His Leu Ser
165 170 175

Pro Arg Val Gly Ala Gln Val Asp Leu Arg Glu Met Lys Leu Leu Leu
180 185 190

Glu Asp Leu Gly Tyr Thr Val Lys Val Lys Glu Asn Leu Thr Ala Leu
195 200 205

Glu Met Val Lys Glu Val Lys Glu Phe Ala Ala Cys Pro Glu His Lys
210 215 220

Thr Ser Asp Ser Thr Phe Leu Val Phe Met Ser His Gly Ile Gln Glu
225 230 235 240

Gly Ile Cys Gly Thr Thr Tyr Ser Asn Glu Val Ser Asp Ile Leu Lys
245 250 255

Val Asp Thr Ile Phe Gln Met Met Asn Thr Leu Lys Cys Pro Ser Leu
260 265 270

Lys Asp Lys Pro Lys Val Ile Ile Ile Gln Ala Cys Arg Gly Glu Lys
275 280 285

Gln Gly Val Val Leu Leu Lys Asp Ser Val Arg Asp Ser Glu Glu Asp
290 295 300

Phe Leu Thr Asp Ala Ile Phe Glu Asp Asp Gly Ile Lys Lys Ala His
305 310 315 320

Ile Glu Lys Asp Phe Ile Ala Phe Cys Ser Ser Thr Pro Asp Asn Val
325 330 335

Ser Trp Arg His Pro Val Arg Gly Ser Leu Phe Ile Glu Ser Leu Ile
340 345 350

Lys His Met Lys Glu Tyr Ala Trp Ser Cys Asp Leu Glu Asp Ile Phe
355 360 365

Arg Lys Val Arg Phe Ser Phe Glu Gln Pro Glu Phe Arg Leu Gln Met
370 375 380

Pro Thr Ala Asp Arg Val Thr Leu Thr Lys Arg Phe Tyr Leu Phe Pro
385 390 395 400

Gly His

CLAIMS

1. An inhibitor of the activity of the *ced-3* gene, comprising a portion of the *ced-3* gene.

2. The inhibitor of Claim 1, wherein the gene portion is a portion of the nucleotide sequence of Figure 3 (SEQ ID NO:1), selected from the group consisting of:
   a) nucleotides 1 to approximately 5850;
   b) nucleotides 1 to approximately 3020; and
   c) an inhibitory subportion (a) and (b).

3. The inhibitor of Claim 1, wherein the gene portion encodes an amino acid sequence of the Ced-3 protein shown in Figures 6A-B (SEQ ID NO:2), selected from the group consisting of:
   a) amino acids 1 to approximately 372;
   b) amino acids 1 to approximately 149; and
   c) an inhibitory subportion of (a) and (b).

4. The inhibitor of Claim 1, further comprising a heterologous structural gene fused 3′ of the gene portion.

5. The inhibitor of Claim 4, wherein the structural gene is *E. coli lacZ*.

6. The inhibitor of Claim 1, further comprising a transcriptional signal and a translational signal suitable for expression of the gene portion in a host cell.

7. The inhibitor of Claim 6, wherein the transcriptional signal and the translational signal are those of the *ced-3* gene.

8. An inhibitor of the activity of the *ced-3* gene, comprising RNA encoded by the sense strand of a nucleotide sequence of Figure 3 (SEQ ID NO:1), the nucleotide sequence selected from the group consisting of:
   a) nucleotides 1 to approximately 5850;
   b) nucleotides 1 to approximately 3020; and
   c) an inhibitory subportion of (a) and (b).

9. An inhibitor of the activity of the *ced-3* gene, comprising protein having an amino acid sequence of the Ced-3 protein shown in Figures 6A-B (SEQ ID NO:2), selected from the group consisting of:
   a) amino acids 1 to approximately 372;
   b) amino acids 1 to approximately 149; and
   c) an inhibitory subportion of (a) and (b).

10. An inhibitor of the activity of the *ced-3* gene, comprising a non-peptide mimetic of the inhibitor of Claim 9.

11. The inhibitor of Claim 1, consisting essentially of a construct selected from BGAFQ and PBA.

12. The inhibitor of Claim 1, comprising the encoded product of a construct selected from BGAFQ and PBA.

13. The inhibitor of Claim 1, comprising a non-peptide mimetic of the protein encoded by a construct selected from BGAFQ and PBA.

14. An inhibitor of the activity of the *ced-3* gene, comprising protein having an amino acid sequence of ICE shown in Figures 6A-B (SEQ ID NO:4), selected from the group consisting of:
a) amino acids 1 to 298;
b) amino acids 1 to 111; and
c) an inhibitory subportion of (a) and (b).

15. An inhibitor of the activity of the *ced-3* gene, comprising a portion of the ICE gene which encodes the protein of Claim 14, or an inhibitory subportion of said gene.

16. An inhibitor of the activity of the *ced-3* gene, comprising RNA encoded by the gene portion of Claim 15.

17. An inhibitor of the activity of the *ced-3* gene, comprising a non-peptide mimetic of the protein of Claim 14.

18. An inhibitor of the activity of the *ced-3* gene, comprising a portion of the protein product of a gene which is structurally related to the *ced-3* gene, said protein portion corresponding to an amino acid sequence of the Ced-3 protein shown in Figures 6A-B (SEQ ID NO:2), selected from the group consisting of:
a) amino acids 1 to approximately 372;

b) amino acids 1 to approximately 149; and
c) an inhibitory subportion of (a) and (b).

19. An inhibitor of the activity of the *ced-3* gene, comprising a portion of a gene which is structurally related to the *ced-3* gene, said gene portion encoding the protein of Claim 18 or an inhibitory subsection of said gene portion.

20. An inhibitor of the activity of the *ced-3* gene, comprising RNA encoded by the gene portion of Claim 19.

21. An inhibitor of the activity of the *ced-3* gene, comprising a non-peptide mimetic of the protein portion of Claim 18.

22. An inhibitor of the activity of the ICE gene comprising a portion of said gene which encodes an amino sequence of ICE shown in Figures 6A-B (SEQ ID NO:4), selected from the group consisting of:
a) amino acids 1 to approximately 298;
b) amino acids 1 to approximately 111; and
c) an inhibitory subportion of (a) and (b).

23. The inhibitor of Claim 22, further comprising a heterologous structural gene fused 3′ of the gene portion.

24. The inhibitor of Claim 22, further comprising a transcriptional signal and a translational signal suitable for expression of the gene portion in a host cell.

25. An inhibitor of the activity of the ICE gene, comprising RNA encoded by the gene portion of Claim 22.

26. An inhibitor of the activity of the ICE gene, comprising an amino acid sequence of ICE shown in Figures 6A-B (SEQ ID NO:4), selected from the group consisting of:
a) amino acids 1 to approximately 298;
b) amino acids 1 to approximately 111; and
c) an inhibitory subportion of (a) and (b).

27. An inhibitor of the activity of the ICE gene, comprising a non-peptide mimetic of the protein of Claim 26.

28. An inhibitor of the activity of the ICE gene, comprising a portion of the *ced-3* gene.

29. The inhibitor of Claim 28, wherein said gene portion is a nucleotide sequence of Figure 3 (SEQ ID NO:1), selected from the group consisting of:
a) nucleotides 1 to approximately 5850;
b) nucleotides 1 to approximately 3020; and
c) an inhibitory subportion of (a) and (b).

30. The inhibitor of Claim 28, wherein the gene portion encodes an amino acid sequence of the Ced-3 protein shown in Figures 6A-B (SEQ ID NO:2), selected from the group consisting of:
a) amino acids 1 to approximately 372;
b) amino acids 1 to approximately 149; and
c) an inhibitory subportion of (a) and (b).

31. The inhibitor of Claim 28, further comprising a heterologous structural gene fused 3′ of the gene portion.

32. The inhibitor of Claim 28, further comprising a transcriptional signal and a translational signal suitable for expression of the gene portion in a host cell.

33. An inhibitor of the activity of the ICE gene, comprising RNA encoded by the sense strand of a portion of the *ced-3* gene, said gene portion which is a nucleotide sequence of Figure 3 (SEQ ID NO:1), selected from the group consisting of:
a) nucleotides 1 to approximately 5850;
b) nucleotides 1 to approximately 3020; and
c) an inhibitory subportion of (a) and (b).

34. An inhibitor of the activity of the ICE gene, comprising protein having an amino acid sequence of the Ced-3 protein shown in Figures 6A-B (SEQ ID NO:4), selected from the group consisting of:
a) amino acids 1 to approximately 372;
b) amino acids 1 to approximately 149; and
c) an inhibitory subportion of (a) and (b).

35. An inhibitor of the activity of the ICE gene, comprising a non-peptide mimetic of the protein of Claim 34.

36. The inhibitor of Claim 28, consisting essentially of a construct selected from BGAFQ and PBA.

37. An inhibitor of the activity of the ICE gene, comprising the encoded product of a construct selected from BGAFQ and PBA.

38. An inhibitor of the activity of the ICE gene comprising a portion of the protein product of a gene which is structurally related to said ICE gene, said protein portion corresponding to an amino acid sequence of the Ced-3 protein shown in Figures 6A-B (SEQ ID NO:2), selected from the group consisting of:
    a) amino acids 1 to approximately 372;
    b) amino acids 1 to approximately 149; and
    c) an inhibitory subportion of (a) and (b).

39. An inhibitor of the activity of the ICE gene, comprising a portion of a gene which is structurally related to the ICE gene, said gene portion encoding the protein of Claim 38, or an inhibitory subsection of said gene portion.

40. An inhibitor of the activity of the ICE gene, comprising RNA encoded by the gene portion of Claim 39.

41. An inhibitor of the activity of the ICE gene, comprising a non-peptide mimetic of the protein of Claim 38.

42. An inhibitor of the activity of a gene belonging to the *ced-3*/ICE family of structurally related genes, comprising DNA selected from the group consisting of:

a) a portion of the nucleotide sequence of Figure 3 (SEQ ID NO:1), selected from the group consisting of:
   1) nucleotides 1 to approximately 5850;
   2) nucleotides 1 to approximately 3020; and
   3) an inhibitory subportion of (a.1) and (a.2);

b) DNA encoding an amino acid sequence of the Ced-3 protein shown in Figures 6A-B (SEQ ID NO:2), selected from the group consisting of:
   1) amino acids 1 to approximately 372;
   2) amino acids 1 to approximately 149; and
   3) an inhibitory subportion of (b.1) and (b.2);

c) a portion of the ICE gene which encodes an amino acid sequence of ICE shown in Figures 6A-B (SEQ ID NO:4), selected from the group consisting of:
   1) amino acids 1 to approximately 298;
   2) amino acids 1 to approximately 111; and
   3) an inhibitory subportion of (c.1) and c.2);

d) a portion of said *ced-3*/ICE gene which encodes an amino acid sequence corresponding to a portion of the Ced-3 protein shown in Figures 6A-B (SEQ ID NO:2), said Ced-3 portion selected from the group consisting of:
   1) amino acids 1 to approximately 372;
   2) amino acids 1 to approximately 149; and
   3) an inhibitory subportion of (d.1) and (d.2); and

e) a portion of a *ced-3*/ICE gene other than said *ced-3*/ICE gene which encodes an amino acid sequence corresponding to a portion of the Ced-3 protein shown in Figures 6A-B (SEQ ID NO:2), said Ced-3 portion selected from the group consisting of:
   1) amino acids 1 to approximately 372;
   2) amino acids 1 to approximately 149; and
   3) an inhibitory subportion of (e.1) and (e.2).

43. An inhibitor of the activity of a gene belonging to the *ced-3*/ICE family of structurally related genes, comprising RNA encoded by the DNA of Claim 42.

44. An inhibitor of the activity of a gene belonging to the *ced-3*/ICE family of structurally related genes, comprising protein encoded by the DNA of Claim 42.

45. An inhibitor of the activity of a gene belonging to the *ced-3*/ICE family of structurally related genes, comprising a non-peptide mimetic of the protein of Claim 44.

46. A drug for reducing cell deaths, comprising an inhibitor of the activity of the *ced-3* gene, selected from the group consisting of:
   a) a portion of the *ced-3* gene;
   b) a product encoded by a portion of the *ced-3* gene;
   c) a non-peptide mimetic of an inhibitory portion of the Ced-3 protein;
   d) a portion of the ICE gene;

e) a product encoded by a portion of the ICE gene;

f) a non-peptide mimetic of an inhibitory portion of the ICE protein;

g) a portion of a gene which is structurally related to the *ced-3* gene;

h) a product encoded by the gene portion of (g); and

i) a non-peptide mimetic of the protein encoded by the gene portion of (g).

47. The drug of Claim 46, wherein the inhibitor is selected from the group consisting of:

a) DNA having a nucleotide sequence of Figure 3 (SEQ ID NO:1), selected from the group consisting of:

1) nucleotides 1 to approximately 5850;

2) nucleotides 1 to approximately 3020; and

3) an inhibitory portion of (a.1) and (a.2);

b) DNA encoding an amino acid sequence of the Ced-3 protein shown in Figures 6A-B (SEQ ID NO:2), selected from:

1) amino acids 1 to approximately 372;

2) amino acids 1 to approximately 149; and

3) an inhibitory portion of (b.1) and (b.2);

c) RNA encoded by DNA of (a);

d) RNA encoded by DNA of (b);

e) protein having an amino acid sequence of the Ced-3 protein shown in Figures 6A-B (SEQ ID NO:2), selected from the group consisting of:

1) amino acids 1 to approximately 372;

2) amino acids 1 to approximately 149; and

3) inhibitory portion of (e.1) and (e.2); and

f) a non-peptide mimetic of the protein of e).

48. The drug of Claim 46, wherein the inhibitor is selected from the group consisting of:

a) DNA encoding an amino acid sequence of ICE shown in Figures 6A-B (SEQ ID NO:4), selected from the group consisting of:

1) amino acids 1 to approximately 298;

2) amino acids 1 to approximately 111; and

3) an inhibitory portion of (a.1) and (a.2);

b) RNA encoded by DNA of a);

c) protein having an amino acid sequence of ICE shown in Figures 6A-B (SEQ ID NO:4), selected from the group consisting of:

1) amino acids 1 to approximately 298;

2) amino acids 1 to approximately 111; and

3) an inhibitory portion of (c.1) and (c.2); and

d) a non-peptide mimetic of the protein of c).

49. The drug of Claim 46, wherein the inhibitor is selected from the group consisting of:

a) protein encoded by a portion of a gene which is structurally related to the *ced-3* gene, said protein portion corresponding to an amino acid sequence of the Ced-3 protein shown in Figures 6A-B (SEQ ID NO:2) selected from the group consisting of:

1) amino acids 1 to approximately 372;

2) amino acids 1 to approximately 149; and

3) an inhibitory portion of (a.1) and (a.2);

b) DNA encoding the protein of (a) or inhibitory subportion thereof;

c) RNA encoding the protein of (a) or inhibitory subportion thereof; and

d) a non-peptide mimetic of the protein of (a).

50. An inhibitor as defined in Claim 46, for use in therapy e.g. for treating a condition characterized by cell deaths.

51. A method for treating a condition characterized by cell deaths, comprising administering the drug of Claim 46.

52. A drug for reducing cell deaths, comprising an inhibitor of the activity of the ICE gene, selected from the group consisting of:

a) a portion of the ICE gene;

b) a product encoded by a portion of the ICE gene;

c) a non-peptide mimetic of an inhibitory portion of the ICE protein;

d) a portion of the *ced-3* gene;

e) a product encoded by a portion of the *ced-3* gene;

f) a non-peptide mimetic of an inhibitory portion of the Ced-3 protein;

g) a portion of a gene which is structurally related to the *ced-3* gene and the ICE gene;

h) a product encoded by the gene portion of (e); and

i) a non-peptide mimetic of the protein encoded by (g).

53. A drug for reducing cell deaths, comprising an inhibitor of the activity of a gene which is structurally related to the *ced-3* gene and ICE gene, selected from the group consisting of:
    a) a portion of said related gene;
    b) a product encoded by the gene portion of (e);
    c) a non-peptide mimetic of the protein product encoded by (a);
    d) a portion of the ICE gene;
    e) a product encoded by the gene portion of (d);
    f) a non-peptide mimetic of a protein product encoded by (d);
    g) a portion of the *ced-3* gene;
    h) a product encoded by the gene portion of (g); and
    i) a non-peptide mimetic of the protein product encoded by (g).

54. A drug for inhibiting the activity of a gene selected from the group consisting of *ced-3* and a gene which belongs to the *ced-3*/ICE gene family, comprising an inhibitor of interleukin-1β convertase.

55. The drug of Claim 54 which reduces cell deaths.

56. The drug of Claim 54 which is a peptide aldehyde containing the amino acid sequence Tyr-Val-X-Asp, wherein X is selected from Ala, His, Gln, Lys, Phe, Cha, and Asp.

57. The drug of Claim 54 which is Ac-Tyr-Val-Ala-Asp-CHO, also referred to as inhibitor B.

58. The drug of Claim 54 which is the cowpox virus CrmA protein.

59. An anti-inflammatory drug, comprising an inhibitor of the activity of the ICE gene, or inhibitory portion thereof, selected from the group consisting of:
   a) a portion of the ICE gene;
   b) a product encoded by a portion of the ICE gene;
   c) a portion of the *ced-3* gene;
   d) a product encoded by a portion of the *ced-3* gene;
   e) a portion of a gene which is structurally related to the *ced-3* gene and ICE gene; and
   f) a product encoded by a portion of a gene which is structurally related to the *ced-3* gene and the ICE gene.

60. The anti-inflammatory drug of Claim 59, wherein the inhibitor is selected from the group consisting of:
   a) DNA encoding an amino acid sequence of ICE shown in Figures 6A-B (SEQ ID NO:4), selected from the group consisting of:
      1) amino acids 1 to approximately 298;
      2) amino acids 1 to approximately 111; and
      3) an inhibitory portion of (a.1) and (a.2);
   b) RNA encoded by DNA of (a) or an inhibitory subportion thereof;
   c) protein having an amino acid sequence of ICE shown in Figures 6A-B (SEQ ID NO:4), selected from the group consisting of:
      1) amino acids 1 to approximately 298;

2) amino acids 1 to approximately 111; and
3) an inhibitory portion of (c.1) and (c.2);

d) a non-peptide mimetic of the protein of (c).

61. The anti-inflammatory drug of Claim 59, wherein the inhibitor is selected from the group consisting of:

a) DNA having a nucleotide sequence of Figure 3 (SEQ ID NO:1), selected from the group consisting of:
1) nucleotides 1 to approximately 5850;
2) nucleotides 1 to approximately 3020; and
3) an inhibitory portion of (a.1) and (a.2);

b) DNA encoding an amino acid sequence of the Ced-3 protein shown in Figures 6A-B (SEQ ID NO:2), selected from the group consisting of:
1) amino acids 1 to approximately 372;
2) amino acids 1 to approximately 149; and
3) an inhibitory portion of (b.1) and (b.2);

c) RNA encoded by DNA of (a);

d) RNA encoded by DNA of (b);

e) protein having an amino acid sequence of the Ced-3 protein shown in Figures 6A-B (SEQ ID NO:2), selected from the group consisting of:
1) amino acids 1 to approximately 372;
2) amino acids 1 to approximately 149; and
3) an inhibitory portion of (e.1) and (e.2); and

f) a non-peptide mimetic of the protein of (e).

62. The anti-inflammatory drug of Claim 59, wherein the inhibitor is selected from the group consisting of:

a) protein encoded by a portion of a gene which is structurally related to the *ced-3* and ICE genes, said protein portion corresponding to an amino acid sequence of ICE shown in Figures 6A-B (SEQ ID NO:4), selected from the group consisting of:
   1) amino acids 1 to approximately 298;
   2) amino acids 1 to approximately 111; and
   3) an inhibitory portion of (a.1) and (a.2);

b) DNA encoding the protein of (a);

c) RNA encoding the protein of (a); and

d) a non-peptide mimetic of the protein of (a).

63. A method for treating inflammation, comprising administering the drug of Claim 59.

64. An inhibitor or inhibitory portion as defined in Claim 59, for use in therapy e.g. for treating inflammation.

65. A method for altering the occurrence of cell death, comprising altering the activity of a cell death gene which is structurally related to *ced-3*.

66. The method of Claim 65, wherein the structurally related gene is ICE.

67. A drug for increasing cell deaths, comprising a molecule, of active portion thereof, selected from:

a) DNA comprising a gene which belongs to the *ced-3*/ICE gene family;

b) RNA encoded by the DNA of (a);

c) protein encoded by the DNA of (a);

d) an agent which is structurally similar to and mimics the activity of the protein of (c);

e) an agonist of the activity of a gene which belongs to the *ced-3*/ICE gene family;

f) DNA comprising a constitutively activated mutated form of a gene which belongs to the *ced-3*/ICE gene family;

g) RNA encoded by the DNA of (e);

h) protein encoded by the DNA of (e);

i) an agent which is structurally similar to and mimics the activity of a protein encoded by the DNA of (e); and

j) an agonist of the activity of a constitutively activated mutated form of a gene which belongs to the *ced-3*/ICE gene family.

68. The drug of Claim 67, wherein the gene which belongs to the *ced-3*/ICE gene family is ICE.

69. The drug of Claim 67(f), wherein the constitutively activated mutated form of the gene which belongs to the *ced-3*/ICE gene family encodes a carboxyl-terminal portion of a protein product of the wild-type gene, said carboxyl-terminal portion having a deletion of an amino-terminal portion which corresponds to an amino acid sequence of the Ced-3 protein shown in Figures 6A-B (SEQ ID NO:2), selected from the group consisting of:

a) amino acids 1 to approximately 372;

b) amino acids 1 to approximately 149; and

c) an inhibitory subportion of (a) and (b).

70. The drug of Claim 69, wherein the protein product of the wild-type gene has sequences corresponding to the autocleavage sites of ICE and the protein product of the wild-type gene is selected from the group consisting of:
    a) the uncleaved form of the protein product; and
    b) the subunits corresponding to the active subunits of ICE.

71. A method for reducing the proliferative capacity or size of a population of cells, comprising contacting the cells with the drug of Claim 67 under conditions suitable for activity of the drug.

72. The method of Claim 71, wherein the population of cells is selected from the group consisting of:
    a) cancerous cells;
    b) cells which produce autoreactive antibodies;
    c) infected cells;
    d) hair follicle cells;
    e) cells which are critical to the life of a parasite;
    f) cells which are critical to the life of a pest; and
    g) cells which are critical to the life of a recombinant organism.

73. A molecule or active portions thereof as defined in Claim 67, for use in therapy e.g. for reducing the proliferative capacity or size of population of cells, selected for example from the group consisting of:
    a) cancerous cells;

b) cells which produce autoreactive antibodies;
c) infected cells;
d) hair follicle cells;
e) cells which are critical to the life of a parasite;
f) cells which are critical to the life of a pest; and
g) cells which are critical to the life of a recombinant organism.

74. A drug for decreasing cell deaths comprising a molecule selected from the group consisting of:
a) single stranded nucleic acid having all or a portion of the antisense sequence of a gene which is structurally related to *ced-3*, said nucleic acid which is complementary to the mRNA of the gene;
b) DNA which directs the expression of (a);
c) a mutated form of a gene which is structurally related to *ced-3*, does not cause cell death and antagonizes the activity of the wild-type gene; and
d) an antagonist of the activity of a gene which is structurally related to *ced-3*.

75. The drug of Claim 74, wherein the structurally related gene is ICE.

76. A molecule as defined in Claim 74, for use in therapy e.g. for treating, in a human or other animal, a condition characterized by cell deaths, and wherein for example the condition is selected from the group consisting of:

a) myocardial infarction;
b) stroke;
c) degenerative disease;
d) traumatic brain injury;
e) hypoxia;
f) pathogenic infection; and
g) hair loss.

77. A method for treating, in a human or other animal, a condition characterized by cell deaths, comprising administering the drug of Claim 74 to the human or other animal under conditions suitable for activity of the drug.

78. The method of Claim 77, wherein the condition is selected from the group consisting of:
a) myocardial infarction;
b) stroke;
c) degenerative disease;
d) traumatic brain injury;
e) hypoxia;
f) pathogenic infection; and
g) hair loss.

79. A constitutively activated cell death protein comprising an amino acid sequence of the Ced-3 protein shown in Figures 6A-B (SEQ ID NO:2), selected from the group consisting of:
a) the amino acids from approximately 150 to 503;
b) the amino acids from approximately 373 to 503;
c) the amino acids from approximately 150 to 372;
d) (b) and (c) together;
e) an active subportion of (a), (b), and (c); and

f) combinations of these.

80. The constitutively activated cell death protein of Claim 79, further comprising a subportion of the region of Ced-3 from amino acids 1 to 149, as shown in Figures 6A-B (SEQ ID NO:2), said subportion which enhances and does not inhibit the activity of the protein.

81. Isolated nucleic acid encoding the protein of Claim 79.

82. A constitutively activated cell death protein having an amino acid sequence of ICE shown in Figures 6A-B (SEQ ID NO:4), selected from the group consisting of:

a) the amino acids from approximately 111 to 404;
b) the amino acids from approximately 298 to 404;
c) the amino acids from approximately 111 to 297;
d) (b) and (c) together;
e) an active subportion of (a), (b), and (c); and
f) combinations of these.

83. Isolated nucleic acid encoding the protein of Claim 82.

84. A drug for increasing cell deaths, comprising a molecule selected from the protein of Claim 79 or a nucleic acid encoding said protein.

85. A drug for increasing cell deaths, comprising a molecule selected from the protein of Claim 79 or a nucleic acid encoding said protein.

86. Isolated protein having cell death activity and the amino acid sequence of the NEDD-2 protein shown in Figure 7 (SEQ ID NO:13), or an active portion thereof.

87. Isolated nucleic acid encoding the protein of Claim 86.

88. An isolated substrate-specific protease having the amino acid sequence of the Ced-3 protein shown in Figures 6A-B (SEQ ID NO:2).

89. An isolated substrate-specific protease, consisting essentially of a protein product of a gene which is structurally related to the *ced-3* and ICE genes.

90. The protease of Claim 89 which cleaves after aspartate residues.

91. The protease of Claim 89 which is a cysteine protease.

92. Isolated ICE having an alteration which reduces the activity of the enzyme, the alteration selected from the group consisting of:
a) L to F at amino acid 26;
b) G to R at amino acid 65;
c) G to S at amino acid 287;
d) E to termination at amino acid 324;
e) W to termination at amino acid 340;
f) A to V at amino acid 361;
g) E to K at amino acid 390; and
h) T to F at amino acid 393.

93. Isolated DNA which is a mutated ICE gene encoding the altered enzyme of Claim 92.

94. Isolated RNA encoded by the DNA of Claim 93.

95. An isolated gene belonging to the *ced-3*/ICE family of structurally related genes which has a mutation which reduces the activity of the gene, said mutation resulting in an amino acid alteration corresponding to an amino acid alteration of the Ced-3 protein which inactivates the Ced-3 protein.

96. A product of the gene of Claim 95 selected from RNA and protein.

97. Isolated protein which is the NEDD-2 protein having an alteration which inactivates the protein, said alteration selected from the group consisting of:
a) A to V at amino acid 117;
b) E to K at amino acid 483; and
c) S to F at amino acid 486.

98. Isolated nucleic acid encoding the protein of Claim 97.

99. Isolated protein which is structurally similar to Ced-3 and has an alteration at a conserved amino acid corresponding to an amino acid of the Ced-3 protein selected from the group consisting of:
a) Ser 183;
b) Met 234;
c) Arg 242;
d) Leu 246;

e) Ile 247;
f) Ile 248;
g) Asn 250;
h) Phe 253;
i) Arg 259;
j) Gly 261;
k) Asp 265;
l) Gly 277;
m) Tyr 278;
n) Val 280;
o) Lys 283;
p) Asn 285;
q) Leu 286;
r) Thr 287;
s) Met 291;
t) Phe 298;
u) His 304;
v) Asp 306;
w) Ser 307;
x) Leu 310;
y) Val 311;
z) Ser 314;
aa) His 315;
bb) Gly 316;
cc) Ile 321;
dd) Gly 323;
ee) Ile 334;
ff) Asn 339;
gg) Pro 344;
hh) Leu 346;
ii) Lys 349;
jj) Pro 350;
kk) Lys 351;

ll) Gln 356;
mm) Ala 357;
nn) Cys 358;
oo) Arg 359;
pp) Gly 360;
qq) Asp 371;
rr) Asp 414;
ss) Arg 429;
tt) Gly 434;
uu) Ser 435;
vv) Ile 438;
ww) Ala 449;
xx) Val 452;
yy) Leu 488;
aa) Tyr 493; and
aaa) Pro 496.

100. Isolated nucleic acid encoding the protein of Claim 99.

101. A method for identifying a portion of the *ced-3* gene which inhibits the activity of the *ced-3* gene, comprising the steps of:
a) injecting wild-type nematodes with a portion of the *ced-3* gene under conditions suitable for expression of said gene portion; and
b) detecting a decrease in programmed cell deaths,

whereby a decrease in programmed cell deaths is indicative of a portion of the *ced-3* gene which inhibits the activity of said gene.

102. Isolated DNA comprising the inhibitory portion of the *ced-3* gene identified by the method of Claim 101.

103. A method for identifying a portion of a gene which is structurally related to the *ced-3* gene which inhibits the activity of the *ced-3* gene, comprising the steps of:
   a) injecting wild-type nematodes with a portion of the structurally related gene under conditions suitable for expression of said gene portion; and
   b) detecting a decrease in programmed cell deaths,

   whereby a decrease in programmed cell deaths is indicative of a portion of the structurally related gene which inhibits the activity of the *ced-3* gene.

104. Isolated DNA comprising the inhibitory portion of the structurally related gene identified by the method of Claim 103.

105. The DNA of Claim 104, wherein the structurally related gene is the ICE gene.

106. A method for identifying a portion of ICE which inhibits the activity of said enzyme, comprising the steps of:
   a) combining a portion of ICE with ICE and a substrate of ICE under conditions suitable for cleavage of the substrate by ICE; and
   b) detecting a decrease in cleavage of the substrate,

whereby a decrease in cleavage of the substrate is indicative of a portion of ICE which inhibits the activity of said enzyme.

107. An isolated inhibitory portion of the ICE protein identified by the method of Claim 106.

108. Isolated nucleic acid encoding the inhibitory portion of Claim 107.

109. A method for identifying a portion of the protein product of a gene which is structurally related to the *ced-3* and ICE genes, and inhibits the activity of ICE, comprising the steps of:

a) combining a portion of the protein product of a gene which is structurally related to the *ced-3* and ICE genes with ICE and a substrate of ICE under conditions suitable for cleavage of the substrate by ICE; and

b) detecting a decrease in cleavage of the substrate,

whereby a decrease in cleavage of the substrate is indicative of a portion of the protein product of a gene which is structurally related to the *ced-3* and ICE genes and inhibits the activity of ICE.

110. An isolated inhibitory portion identified by the method of Claim 109.

111. Isolated nucleic acid encoding the inhibitory portion of Claim 110.

112. A method for identifying a gene which is structurally related to the *ced-3* gene and the ICE gene, comprising detecting a gene with:
    a) a probe derived from the *ced-3* gene or a product encoded by the *ced-3* gene; and
    b) a probe derived from the ICE gene or a product encoded by the ICE gene.

113. An isolated gene identified by the method of Claim 112.

114. A method for identifying a gene which belongs to the *ced-3*/ICE family of structurally related genes, comprising detecting a gene with a probe selected from the group consisting of:
    a) a probe derived from a gene which is structurally related to the *ced-3* gene and the ICE gene; and
    b) a probe derived from the consensus sequence of a conserved region in genes belonging to the *ced-3*/ICE gene family.

115. An isolated gene identified by the method of Claim 114 which has an activity selected from cell death activity and protease activity.

116. Isolated DNA selected from the group consisting of:
    a) a region of a gene belonging to the *ced-3*/ICE family of structurally related genes which is conserved among two or more family members; and

b) the consensus sequence of a conserved region in genes belonging to the *ced-3*/ICE gene family,

or encoded product thereof.

117. A method for identifying a gene which interacts with a *ced-3*/ICE gene belonging to said family, comprising identifying a mutation which enhances or suppresses the activity of a *ced-3*/ICE gene in a nematode, wherein the enhancing or suppressing mutation is indicative of a gene which interacts with the *ced-3*/ICE gene.

118. The method of Claim 117, wherein the *ced-3*/ICE gene is selected from the group consisting of:

a) a wild-type *ced-3* gene;

b) a mutated *ced-3* gene, the nematode being a mutant nematode;

c) a transgene which is a wild-type form of said *ced-3*/ICE gene, the nematode being a transgenic nematode having an inactivated endogenous *ced-3* gene; and

d) a transgene which is a mutated form of said *ced-3*/ICE gene, the nematode being a transgenic nematode having an inactivated endogenous *ced-3* gene.

119. An isolated gene identified by the method of Claim 117, or an encoded product thereof.

120. A bioassay for identifying an agent which affects the activity of a gene belonging to the *ced-3*/ICE family of structurally related genes, comprising the steps of:
   a) introducing an agent into a transgenic nematode which expresses a *ced-3*/ICE gene; and
   b) detecting an alteration in the occurrence of cell deaths in the transgenic nematode, wherein an alteration indicates that the agent affects the activity of the *ced-3*/ICE gene.

121. The method of Claim 120, wherein the *ced-3*/ICE gene is selected from a wild-type gene and a mutated gene.

122. An agent identified by the method of Claim 120.

123. A diagnostic probe for a disease characterized by cell deaths, comprising a molecule selected from the group consisting of:
   a) all or a portion of the *ced-3* gene (SEQ ID NO:1) which is specific to said *ced-3* gene;
   b) RNA encoded by the *ced-3* gene;
   c) degenerate oligonucleotides derived from the amino acid sequence of the Ced-3 protein (SEQ ID NO:2);
   d) an antibody directed against the Ced-3 protein;
   e) all or a portion of the ICE gene (SEQ ID NO:3) which is specific to said ICE gene;
   f) RNA encoded by the ICE gene;
   g) degenerate oligonucleotides derived from the amino acid sequence of ICE (SEQ ID NO:4);

h) an antibody directed against ICE;

i) a gene which is structurally related to the *ced-3* gene, or portion thereof specific to said structurally related gene;

j) RNA encoded by the structurally related gene;

k) degenerate oligonucleotides derived from the amino acid sequence of the protein product of a gene which is structurally related to *ced-3*; and

d) an antibody directed against the protein product of a gene which is structurally related to *ced-3*.

124. A method for diagnosis of a disease characterized by cell deaths, comprising detecting an abnormality in the sequence of a gene which is structurally related to *ced-3*.

125. The method of Claim 124, wherein the structurally related gene is ICE.

126. A method for diagnosis of a disease characterized by cell deaths, comprising detecting an abnormality in the activity of a gene which is structurally related to *ced-3*.

127. The method of Claim 126, wherein the structurally related gene is ICE.

128. A diagnostic probe for an inflammatory disease, comprising a molecule selected from the group consisting of:

a) all or a portion of the *ced-3* gene shown in Figure 3 (SEQ ID NO:1) which is specific to the *ced-3* gene;

b) RNA encoded by (a);

c) degenerate oligonucleotides derived from the amino acid sequence of the Ced-3 protein as shown in Figures 6A-B (SEQ ID NO:2);

d) an antibody directed against the Ced-3 protein;

e) a gene which is structurally related to the *ced-3* and ICE genes, or portion thereof which is specific for said related gene;

f) RNA encoded by (a);

g) degenerate oligonucleotides derived from the amino acid sequence of the protein encoded by (e); and

h) an antibody directed against the protein encoded by (e).

129. A method for diagnosis of an inflammatory disease, comprising detecting an abnormality in the sequence of a gene which is a member of the *ced-3*/ICE gene family.

130. The method of Claim 129, wherein the gene is *ced-3*.

131. A method for diagnosis of an inflammatory disease, comprising detecting an abnormality in the activity of a gene which belongs to the *ced-3*/ICE gene family, or an encoded product thereof.

132. The method of Claim 131, wherein the gene is *ced-3*.

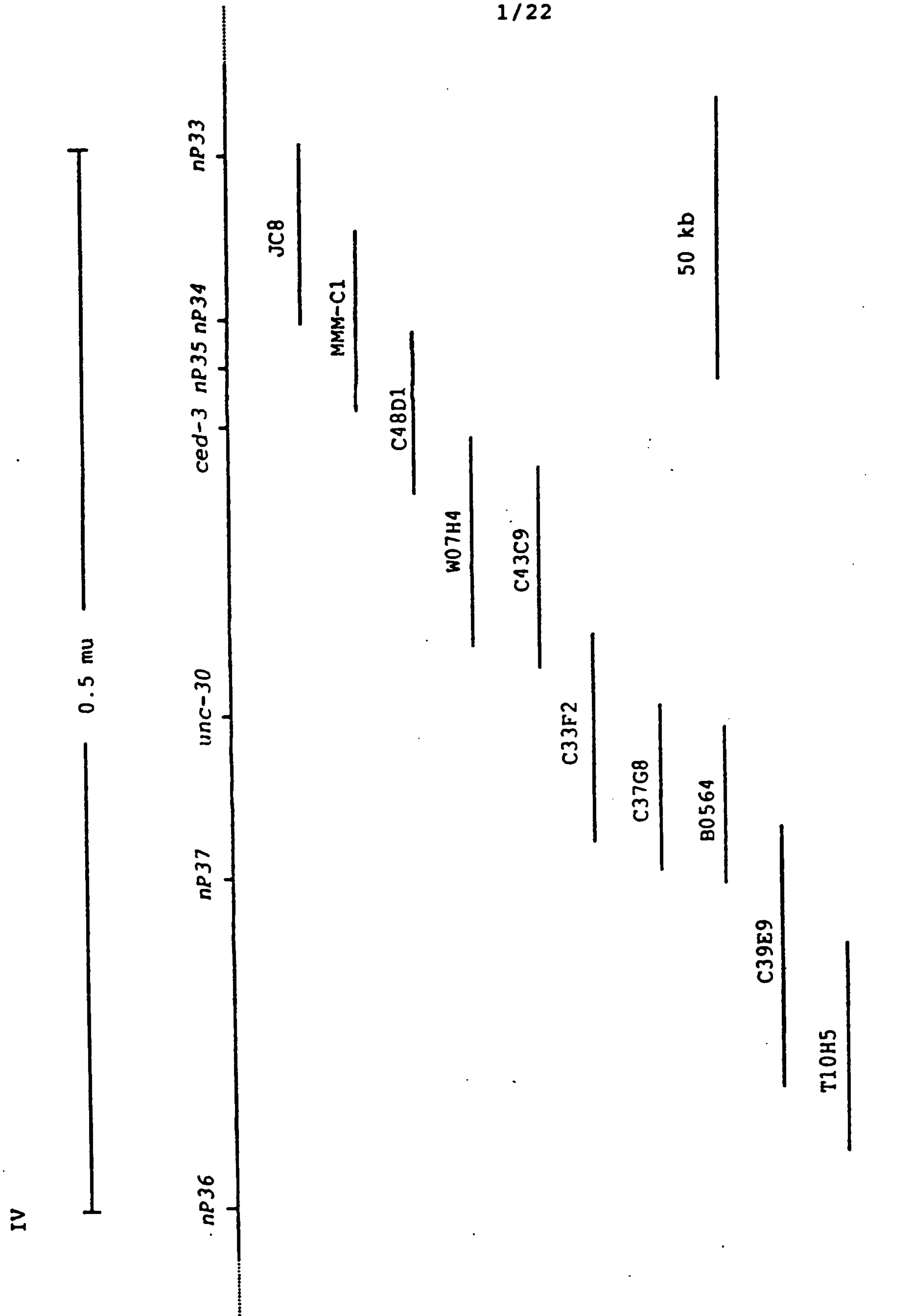
IV
0.5 mu
nP33
nP34
nP35
ced-3
unc-30
nP37
nP36
JC8
MMM-C1
C48D1
W07H4
C43C9
C33F2
C37G8
B0564
C39E9
T10H5
50 kb

FIGURE 1

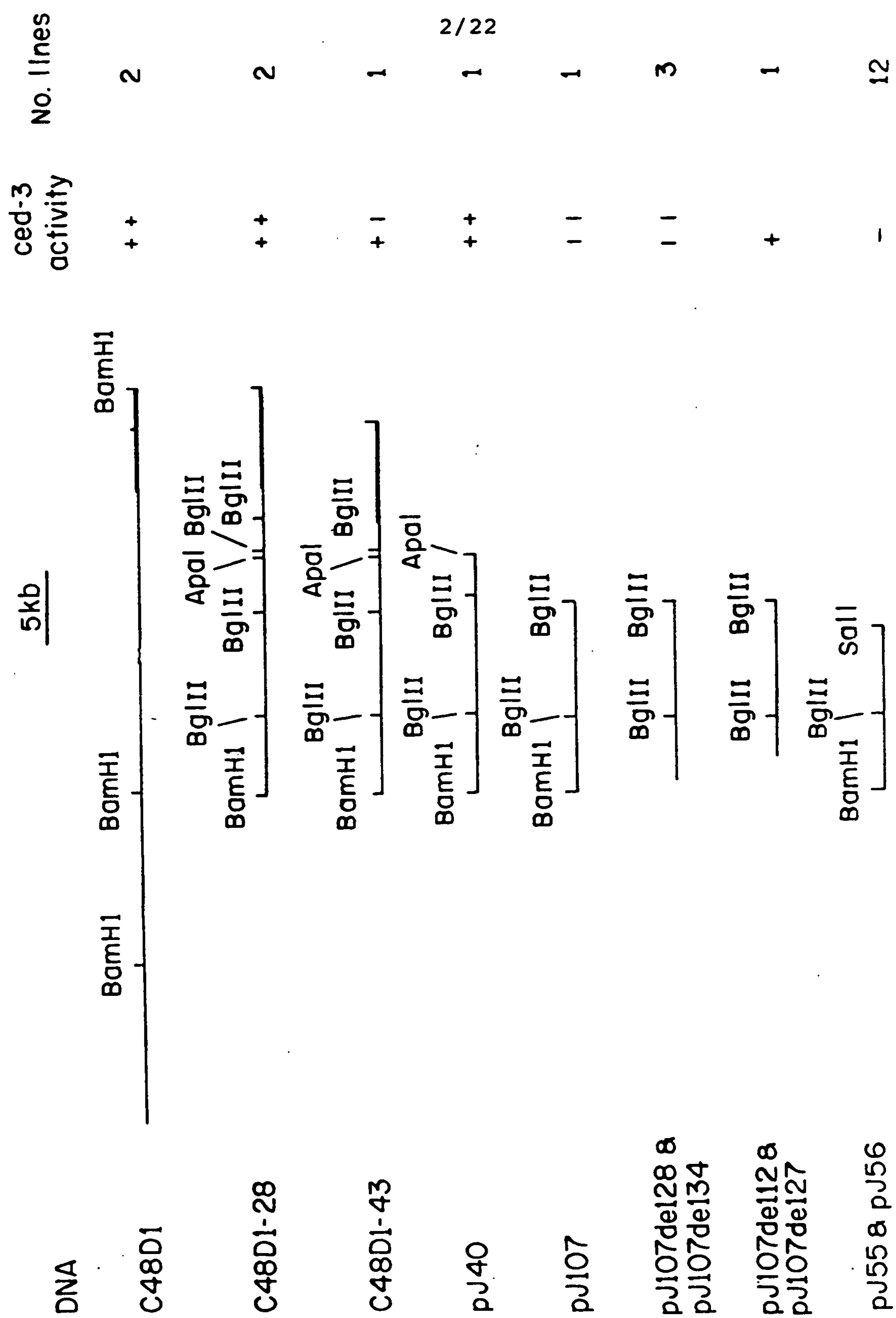
5kb
DNA
ced-3 activity
No. lines
C48D1
BamH1
BamH1
BamH1
++
2
C48D1-28
BamH1
BglII
BglII
ApaI
BglII
BglII
++
2
C48D1-43
BamH1
BglII
BglII
ApaI
BglII
+-
1
pJ40
BamH1
BglII
BglII
ApaI
++
1
pJ107
BamH1
BglII
BglII
--
1
pJ107del28 &
pJ107del34
BglII
BglII
--
3
pJ107del12 &
pJ107del27
BglII
BglII
+
1
pJ55 & pJ56
BamH1
BglII
SalI
-
12

FIG. 2

ced-3 Genomic Sequence

```
1     AGATCTGAAATAAGGTGATAAATTAATAAATTAAGTGTATTTCTGAGGAAATTTGACTGT   60
61    TTTAGCACAATTAATCTTGTTTCAGAAAAAAAGTCCAGTTTTCTAGATTTTTCCGTCTTA   120
121   TTGTCGAATTAATATCCCTATTATCACTTTTTCATGCTCATCCTCGAGCGGCACGTCCTC   180
181   AAAGAATTGTGAGAGCAAACGCGCTCCCATTGACCTCCACACTCAGCCGCCAAAACAAAC   240
241   GTTCGAACATTCGTGTGTTGTGCTCCTTTTCCGTTATCTTGCAGTCATCTTTTGTCGTTT   300
301   TTTTCTTTGTTCTTTTTGTTGAACGTGTTGCTAAGCAATTATTACATCAATTGAAGAAAA   360
361   GGCTCGCCGATTTATTGTTGCCAGAAAGATTCTGAGATTCTCGAAGTCGATTTTATAATA   420
421   TTTAACCTTGGTTTTTGCATTGTTTCGTTTAAAAAAACCACTGTTTATGTGAAAAACGAT   480
481   TAGTTTACTAATAAAACTACTTTTAAACCTTTACCTTTACCTCACCGCTCCGTGTTCATG   540
541   GCTCATAGATTTTCGATACTCAAATCCAAAAATAAATTTACGAGGGCAATTAATGTGAAA   600
601   CAAAAACAATCCTAAGATTTCCACATGTTTGACCTCTCCGGCACCTTCTTCCTTAGCCCC   660
661   ACCACTCCATCACCTCTTTGGCGGTGTTCTTCGAAACCCACTTAGGAAAGCAGTGTGTAT   720
721   CTCATTTGGTATGCTCTTTTCGATTTTATAGCTCTTTGTCGCAATTTCAATGCTTTAAAC   760
781   AATCCAAATCGCATTATATTTGTGCATGGAGGCAAATGACGGGGTTGGAATCTTAGATGA   840
841   GATCAGGAGCTTTCAGGGTAAACGCCCGGTTCATTTTGTACCACATTTCATCATTTTCCT   900
901   GTCGTCCTTGGTATCCTCAACTTGTCCCGGTTTTGTTTTCGGTACACTCTTCCGTGATGC   960
961   CACCTGTCTCCGTCTCAATTATCGTTTAGAAATGTGAACTGTCCAGATGGGTGACTCATA   1020
1021  TTGCTGCTGCTACAATCCACTTTCTTTTCTCATCGGCAGTCTTACGAGCCCATCATAAAC   1080
1081  TTTTTTTTCCGCGAAATTTGCAATAAACCGGCCAAAAACTTTCTCCAAATTGTTACGCAA   1140
1141  TATATACAATCCATAAGAATATCTTCTCAATGTTTATGATTTCTTCGCAGCACTTTCTCT   1200
1201  TCGTGTGCTAACATCTTATTTTTATAATATTTCCGCTAAAATTCCGATTTTTGAGTATTA   1260
1261  ATTTATCGTAAAATTATCATAATAGCACCGAAAACTACTAAAAATGGTAAAAGCTCCTTT   1320

                                                     Repeat 1
1321  TAAATCGGCTCGACATTATCGTATTAAGGAATCACAAAATTCTGAGAATGCGTACTGCGC   1380

1381  AACATATTTGACGGCAAAATATCTCGTAGCGAAAACTACAGTAATTCTTTAAATGACTAC   1440

                                                          Repeat 1
1441  TGTAGCGCTTGTGTCGATTTACGGGCTCAATTTTTGAAAATAATTTTTTTTTTCGAATTT   1500
```

FIGURE 3(a)

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1501  TGATAACCCGTAAATCGTCACAACGCTACAGTAGTCATTTAAAGGATTACTGTAGTTCTA  1560

1561  GCTACGAGATATTTTGCGCGCCAAATATGACTGTAATACGCATTCTCTGAATTTTGTGTT  1620
1621  TCCGTAATAATTTCACAAGATTTTGGCATTCCACTTTAAAGGCGCACAGGATTTATTCCA  1680
1681  ATGGGTCTCGGCACGCAAAAAGTTTGATAGACTTTTAAATTCTCCTTGCATTTTTAATTC  1740
1741  AATTACTAAAATTTTCGTGAATTTTTCTGTTAAAATTTTTAAAATCAGTTTTCTAATATT  1800
1801  TTCCAGGCTGACAAACAGAAACAAAAACACAACAAACATTTTAAAAATCAGTTTTCAAAT  1860
1861  TAAAAATAACGATTTCTCATTGAAAATTGTGTTTTATGTTTGCGAAAATAAAAGAGAACT  1920
1921  GATTCAAAACAATTTTAACAAAAAAAAACCCCAAAATTCGCCAGAAATCAAGATAAAAAA  1980
1981  TTCAAGAGGGTCAAAATTTTCCGATTTTACTGACTTTCACCTTTTTTTTCGTAGTTCAGT  2040
2041  GCAGTTGTTGGAGTTTTTGACGAAAACTAGGAAAAAAATCGATAAAAATTACTCAAATCG  2100
2101  AGCTGAATTTTGAGGACAATGTTTAAAAAAAAACACTATTTTTCCAATAATTTCACTCAT  2160

2161  TTTCAGACTAAATCGAAAATCAAATCGTACTCTGACTACGGGTCAGTAGAGAGGTCAACC  2220

2221  ATCAGCCGAAGATGATGCGTCAAGATAGAAGGAGCTTGCTAGAGAGGAACATTATGATGT  2280
                  M  M  R  Q  D  R  R  S  L  L  E  R  N  I  M  M  F
                  1                          10

                                          T(n1040)
                                          |
2281  TCTCTAGTCATCTAAAAGTCGATGAAATTCTCGAAGTTCTCATCGCAAAACAAGTGTTGA  2340
        S  S  H  L  K  V  D  E  I  L  E  V  L  I  A  K  Q  V  L  N
              20                          30

                                    |intron 1
2341  ATAGTGATAATGGAGATATGATTAATGTGAGTTTTTAATCGAATAATAATTTTAAAAAAA  2400
        S  D  N  G  D  M  I  N
              40

                                         |
2401  AATTGATAATATAAAGAATATTTTTGCAGTCATGTGGAACGGTTCGCGAGAAGAGACGGG  2460
                                         S  C  G  T  V  R  E  K  R  R  E
                                                     50

                                    A(n718)
                                    |
2461  AGATCGTGAAAGCAGTGCAACGACGGGGAGATGTGGCGTTCGACGCGTTTTATGATGCTC  2520
        I  V  K  A  V  Q  R  R  G  D  V  A  F  D  A  F  Y  D  A  L
                 60                          70

                                                           |intron 2
2521  TTCGCTCTACGGGACACGAAGGACTTGCTGAAGTTCTTGAACCTCTCGCCAGATCGTAGG  2580
        R  S  T  G  H  E  G  L  A  E  V  L  E  P  L  A  R  S
                    80                          90
```

FIGURE 3(b)

```
     TTTTTAAAGTTCGGCGCAAAAGCAAGGGTCTCACGGAAAAAAGAGGCGGATCGTAATTTT
2581 ---------+---------+---------+---------+---------+---------+ 2640
     GCAACCCACCGGCACGGTTTTTCCTCCGAAAATCGGAAATTATGCACTTTCCCAAATAT
2641 ---------+---------+---------+---------+---------+---------+ 2700
     TTGAAGTGAAATATATTTTATTTACTGAAAGCTCGAGTGATTATTTATTTTTTAACACTA
2701 ---------+---------+---------+---------+---------+---------+ 2760
     ATTTTCGTGGCGCAAAAGGCCATTTTGTAGATTTGCCGAAAATACTTGTCACACACACAC
2761 ---------+---------+---------+---------+---------+---------+ 2820

                                   |
     ACACACATCTCCTTCAAATATCCCTTTTTCCAGTGTTGACTCGAATGCTGTCGAATTCGA
2821 ---------+---------+---------+---------+---------+---------+ 2880
                                     V  D  S  N  A  V  E  F  E
                                                    100

     GTGTCCAATGTCACCGGCAAGCCATCGTCGGAGCCGCGCATTGAGCCCCGCCGGCTACAC
2881 ---------+---------+---------+---------+---------+---------+ 2940
      C  P  M  S  P  A  S  H  R  R  S  R  A  L  S  P  A  G  Y  T
                        110                           120

     TTCACCGACCCGAGTTCACCGTGACAGCGTCTCTTCAGTGTCATCATTCACTTCTTATCA
2941 ---------+---------+---------+---------+---------+---------+ 3000
      S  P  T  R  V  H  R  D  S  V  S  S  V  S  S  F  T  S  Y  Q
                        130                           140

     GGATATCTACTCAAGAGCAAGATCTCGTTCTCGATCGCGTGCACTTCATTCATCGGATCG
3001 ---------+---------+---------+---------+---------+---------+ 3060
      D  I  Y  S  R  A  R  S  R  S  R  S  R  A  L  H  S  S  D  R
                        150                           160

                                                 | intron 3
     ACACAATTATTCATCTCCTCCAGTCAACGCATTTCCCAGCCAACCTTGTATGTTGATGCG
3061 ---------+---------+---------+---------+---------+---------+ 3120
      H  N  Y  S  S  P  P  V  N  A  F  P  S  Q  P  S
                        170

              Repeat 1
         ====================================================
     AACACTAAATTCTGAGAATGCGCATTACTCAACATATTTGACGCGCAAATATCTCGTAGC
3121 ---------+---------+---------+---------+---------+---------+ 3180

     ============================================================
     GAAAAATACAGTAACCCTTTAAATGACTATTGTAGTGTCGATTTACGGGCTCGATTTTCG
3181 ---------+---------+---------+---------+---------+---------+ 3240

     ==>
     AAACGAATATATGCTCGAATTGTGACAACGAATTTTAATTTGTCATTTTTGTGTTTTCTT
3241 ---------+---------+---------+---------+---------+---------+ 3300

                                     Repeat 1
                              <==============================
     TTGATATTTTTGATCAATTAATAAATTATTTCCGTAAACAGACACCAGCGCTACAGTACT
3301 ---------+---------+---------+---------+---------+---------+ 3360

     ====================================
     CTTTTAAAGAGTTACAGTAGTTTTCGCTTCAAGATATTTTGAAAAGAATTTTAAACATTT
3361 ---------+---------+---------+---------+---------+---------+ 3420
     TGAAAAAAAATCATCTAACATGTGCCAAAACGCTTTTTTCAAGTTTCGCAGATTTTTTGA
3421 ---------+---------+---------+---------+---------+---------+ 3480
```

FIGURE 3(c)

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```
          Repeat 2
     TTTTTTTCATTCAAGATATGCTTATTAACACATATAATTATCATTAATGTGAATTTCTTG
3481 ---------+---------+---------+---------+---------+---------+ 3540

     TAGAAATTTTGGGCTTTTCGTTCTAGTATGCTCTACTTTTGAAATTGCTCAACGAAAAAA
3541 ---------+---------+---------+---------+---------+---------+ 3600

     TCATGTGGTTTGTTCATATGAATGACGAAAAATAGCAATTTTTTATATATTTTCCCCTAT
3601 ---------+---------+---------+---------+---------+---------+ 3660

     TCATGTTGTGCAGAAAAATAGTAAAAAAGCGCATGCATTTTTCGACATTTTTTACATCGA
3661 ---------+---------+---------+---------+---------+---------+ 3720

     ACGACAGCTCACTTCACATGCTGAAGACGAGAGACGCGGAGAAATACCACACATCTTTCT
3721 ---------+---------+---------+---------+---------+---------+ 3780

        Repeat 2
     GCGTCTCTCGTCTTCAGCATGTGAAATGGGATCTCGGTCGATGTAAAAAAATGTCGAATA
3781 ---------+---------+---------+---------+---------+---------+ 3840

     ATGTAAAAAATGCATGCGTTTTTTTACACTTTTCTGCACAAATGAATAGGGGGAAAATGT
3841 ---------+---------+---------+---------+---------+---------+ 3900

     ATTAAAATACATTTTTTTGTATTTTTCAACATCACATGATTAACCCCATTATTTTTTCGTT
3901 ---------+---------+---------+---------+---------+---------+ 3960

     GAGCAACTTAAAAAGTAGAGAATATTAGAGCGAAAACCAAAATTTCTTCAAGATATTACC
3961 ---------+---------+---------+---------+---------+---------+ 4020

     TTTATTGATAATTATAGATGTTAATAAGCATATCTTGAATGAAAGTCAGCAAAAATATGT
4021 ---------+---------+---------+---------+---------+---------+ 4080
     GCGAAACACCTGAAAAAAATCAAAAATTCTGCGAAAATTGAAAAAATGCATTAAAATACA
4081 ---------+---------+---------+---------+---------+---------+ 4140
     TTTTTGCATTTTTCTACATCACATGAATGTAGAAAATTAAAAGGGAAATCAAAATTTCTA
4141 ---------+---------+---------+---------+---------+---------+ 4200
     GAGGATATAATTGAATGAAACATTGCGAAATTAAAATGTGCGAAACGTCAAAAAAGAGGA
4201 ---------+---------+---------+---------+---------+---------+ 4260

                                                |
     AATTTGGGTATCAAAATCGATCCTAAAACCAACACATTTCAGCATCCGCCAACTCTTCAT
4261 ---------+---------+---------+---------+---------+---------+ 4320
                                                S  A  N  S  S  F
                                                180
```

FIGURE 3(d)

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```
     TCACCGGATGCTCTTCTCTCGGATACAGTTCAAGTCGTAATCGCTCATTCAGCAAAGCTT
4321 ---------+---------+---------+---------+---------+---------+ 4380
      T  G  C  S  S  L  G  Y  S  S  S  R  N  R  S  F  S  K  A  S
               190                           200

     CTGGACCAACTCAATACATATTCCATGAAGAGGATATGAACTTTGTCGATGCACCAACCA
4381 ---------+---------+---------+---------+---------+---------+ 4440
      G  P  T  Q  Y  I  F  H  E  E  D  M  N  F  V  D  A  P  T  I
               210                           220

     TAAGCCGTGTTTTCGACGAGAAAACCATGTACAGAAACTTCTCGAGTCCTCGTGGAATGT
4441 ---------+---------+---------+---------+---------+---------+ 4500
      S  R  V  F  D  E  K  T  M  Y  R  N  F  S  S  P  R  G  M  C
               230                           240

     GCCTCATCATAAATAATGAACACTTTGAGCAGATGCCAACACGGAATGGTACCAAGGCCG
4501 ---------+---------+---------+---------+---------+---------+ 4560
      L  I  I  N  N  E  H  F  E  Q  M  P  T  R  N  G  T  K  A  D
               250                           260

     ACAAGGACAATCTTACCAATTTGTTCAGATGCATGGGCTATACGGTTATTTGCAAGGACA
4561 ---------+---------+---------+---------+---------+---------+ 4620
      K  D  N  L  T  N  L  F  R  C  M  G  Y  T  V  I  C  K  D  N
               270                           280

              | intron 4
     ATCTGACGGGAAGGGTACGGCGAAATTATATTACCCAAACGCGAAATTTGCCATTTTGCG
4621 ---------+---------+---------+---------+---------+---------+ 4680
      L  T  G  R


            Repeat 3
         ------------------------------->
     CCGAAAATGTGGCGCCCGGTCTCGACACGACAATTTGTGTTAAATGCAAAAATGTATAAT
4681 ---------+---------+---------+---------+---------+---------+ 4740
     TTTGCAAAAAACAAAATTTTGAACTTCCGCGAAAATGATTTACCTAGTTTCGAAATTTTC
4741 ---------+---------+---------+---------+---------+---------+ 4800
     GTTTTTTCCGGCTACATTATGTGTTTTTTCTTAGTTTTTCTATAATATTTGATGTAAAAA
4801 ---------+---------+---------+---------+---------+---------+ 4860
     ACCGTTTGTAAATTTTCAGACAATTTTCCGCATACAAAACTTGATAGCACGAAATCAATT
4861 ---------+---------+---------+---------+---------+---------+ 4920
     TTCTGAATTTTCAAAATTATCCAAAAATGCACAATTTAAAATTTGTGAAAATTGGCAAAC
4921 ---------+---------+---------+---------+---------+---------+ 4980
     GGTGTTTCAATATGAAATGTATTTTTAAAAACTTTAAAAACCACTCCGGAAAAGCAATAA
4981 ---------+---------+---------+---------+---------+---------+ 5040
     AAATCAAAACAACGTCACAATTCAAATTCAAAAGTTATTCATCCGATTTGTTTATTTTTG
5041 ---------+---------+---------+---------+---------+---------+ 5100
     CAAAATTTGAAAAAATCATGAAGGATTTAGAAAAGTTTTATAACATTTTTTCTAGATTTT
5101 ---------+---------+---------+---------+---------+---------+ 5160
     TCAAAATTTTTTTTAACAAATCGAGAAAAAGAGAATGAAAAATCGATTTTAAAAATATCC
5161 ---------+---------+---------+---------+---------+---------+ 5220

       Repeat 3
     <-----------------------------------------------------------
     ACAGCTTCGAGAGTTTGAAATTACAGTACTCCTTAAAGGCGCACACCCCATTTGCATTGG
5221 ---------+---------+---------+---------+---------+---------+ 5280

     --------------------------------------------------
     ACCAAAAATTTGTCGTGTCGAGACCAGGTACCGTAGTTTTTGTCGCAAAAATTGCACCAT
5281 ---------+---------+---------+---------+---------+---------+ 5340
     TGGACAATAAACCTTCCTAATCACCAAAAAGTAAAATTGAAATCTTCGAAAAGCCAAAAA
5341 ---------+---------+---------+---------+---------+---------+ 5400
```

FIGURE 3(e)

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```
     ATTCAAAAAAAAAAGTCGAATTTCGATTTTTTTTTTGGTTTTTTGGTCCCAAAAACCAAAA
5401 ---------+---------+---------+---------+---------+---------+ 5460
     AAATCAATTTTCTGCAAAATACCAAAAAGAAACCCGAAAAAATTTCCCAGCCTTGTTCCT
5461 ---------+---------+---------+---------+---------+---------+ 5520
                                   |
     AATGTAAACTGATATTTAATTTCCAGGGAATGCTCCTGACAATTCGAGACTTTGCCAAAC
5521 ---------+---------+---------+---------+---------+---------+ 5580
                                 G  M  L  L  T  I  R  D  F  A  K  H
                                 290                             300

     ACGAATCACACGGAGATTCTGCGATACTCGTGATTCTATCACACGGAGAAGAGAATGTGA
5581 ---------+---------+---------+---------+---------+---------+ 5640
       E  S  H  G  D  S  A  I  L  V  I  L  S  H  G  E  E  N  V  I
                               310                           320

     TTATTGGAGTTGATGATATACCGATTAGTACACACGAGATATATGATCTTCTCAACGCGG
5641 ---------+---------+---------+---------+---------+---------+ 5700
       I  G  V  D  D  I  P  I  S  T  H  E  I  Y  D  L  L  N  A  A
                               330                           340

                                                           A(n2433)
                                                           |  | intron 5
     CAAATGCTCCCCGTCTGGCGAATAAGCCGAAAATCGTTTTTGTGCAGGCTTGTCGAGGCG
5701 ---------+---------+---------+---------+---------+---------+ 5760
       N  A  P  R  L  A  N  K  P  K  I  V  F  V  Q  A  C  R  G  E
                               350                           360

                                                           |
     GTTCGTTTTTTATTTTAATTTTAATATAAATATTTTAAATAAATTCATTTTCAGAACGTC
5761 ---------+---------+---------+---------+---------+---------+ 5820
                                                            R  R

     GTGACAATGGATTCCCAGTCTTGGATTCTGTCGACGGAGTTCCTGCATTTCTTCGTCGTG
5821 ---------+---------+---------+---------+---------+---------+ 5880
       D  N  G  F  P  V  L  D  S  V  D  G  V  P  A  F  L  R  R  G
                         370                           380

                                                            T(n1165)
                                                            |
     GATGGGACAATCGAGACGGGCCATTGTTCAATTTTCTTGGATGTGTGCGGCCGCAAGTTC
5881 ---------+---------+---------+---------+---------+---------+ 5940
       W  D  N  R  D  G  P  L  F  N  F  L  G  C  V  R  P  Q  V  Q
                         390                           400

     | intron 6
     AGGTTGCAATTTAATTTCTTGAATGAGAATATTCCTTCAAAAAATCTAAAATAGATTTTT
5941 ---------+---------+---------+---------+---------+---------+ 6000
     ATTCCAGAAAGTCCCGATCGAAAAATTGCGATATAATTACGAAATTTGTGATAAAATGAC
6001 ---------+---------+---------+---------+---------+---------+ 6060

          Repeat 4
       ----------------------------------------------------------
     AAACCAATCAGCATCGTCGATCTCCGCCCACTTCATCGGATTGGTTTGAAAGTGGGCGGA
6061 ---------+---------+---------+---------+---------+---------+ 6120

     ----------------->
     GTGAATTGCTGATTGGTCGCAGTTTTCAGTTTAGAGGGAATTTAAAAATCGCCTTTTCGA
6121 ---------+---------+---------+---------+---------+---------+ 6180
     AAATTAAAAATTGATTTTTTCAATTTTTTCGAAAAATATTCCGATTATTTTATATTCTTT
6181 ---------+---------+---------+---------+---------+---------+ 6240
```

FIGURE 3(f)

```
                                                                    A(n717)
                                                                      |
       GGAGCGAAAGCCCCGTCCTGTAAACATTTTTAAATGATAATTAATAAATTTTTGCAGCAA
  6241 ---------+---------+---------+---------+---------+---------+  6300
                                                                      Q

                         T(n1949)
                            |
       GTGTGGAGAAAGAAGCCGAGCCAAGCTGACATTCTGATTCGATACGCAACGACAGCTCAA
  6301 ---------+---------+---------+---------+---------+---------+  6360
       V  W  R  K  K  P  S  Q  A  D  I  L  I  R  Y  A  T  T  A  Q
                      410                          420

                A(n1286)
                   |
       TATGTTTCGTGGAGAAACAGTGCTCGTGGATCATGGTTCATTCAAGCCGTCTGTGAAGTG
  6361 ---------+---------+---------+---------+---------+---------+  6420
       Y  V  S  W  R  N  S  A  R  G  S  W  F  I  Q  A  V  C  E  V
                      430                          440

                 T(n1129,n1164)
                   |
       TTCTCGACACACGCAAAGGATATGGATGTTGTTGAGCTGCTGACTGAAGTCAATAAGAAG
  6421 ---------+---------+---------+---------+---------+---------+  6480
       F  S  T  H  A  K  D  M  D  V  V  E  L  L  T  E  V  N  K  K
                      450                          460

          T(n2430)                                              A(n2426)
            |                                                   | | intron 7
       GTCGCTTGTGGATTTCAGACATCACAGGGATCGAATATTTTGAAACAGATGCCAGAGGTA
  6481 ---------+---------+---------+---------+---------+---------+  6540
       V  A  C  G  F  Q  T  S  Q  G  S  N  I  L  K  Q  M  P  E
                      470                          480

                                            Repeat 5
                                  ==========================
       CTTGAAACAAACAATGCATGTCTAACTTTTAAGGACACAGAAAAATAGGCAGAGGCTCCT
  6541 ---------+---------+---------+---------+---------+---------+  6600

       ========================>
       TTTGCAAGCCTGCCGCGCGTCAACCTAGAATTTTAGTTTTTAGCTAAAATGATTGATTTT
  6601 ---------+---------+---------+---------+---------+---------+  6660
       GAATATTTTATGCTAATTTTTTTGCGTTAAATTTTGAAATAGTCACTATTTATCGGGTTT
  6661 ---------+---------+---------+---------+---------+---------+  6720
       CCAGTAAAAAAATGTTTATTAGCCATTGGATTTTACTGAAAACGAAAATTTGTAGTTTTTC
  6721 ---------+---------+---------+---------+---------+---------+  6780
       AACGAAATTTATCGATTTTTAAATGTAAAAAAAAATAGCGAAAATTACATCAACCATCAA
  6781 ---------+---------+---------+---------+---------+---------+  6840
       GCATTTAAGCCAAAATTGTTAACTCATTTAAAAATTAATTCAAAGTTGTCCACGAGTATT
  6841 ---------+---------+---------+---------+---------+---------+  6900

                  Repeat 5
          <=================================================
       ACACGGTTGGCGCGCGGCAAGTTTGCAAAACGACGCTCCGCCTCTTTTTCTGTGCGGCTT
  6901 ---------+---------+---------+---------+---------+---------+  6960

                                                                  T(n1163)
                                                            |     |
       =====
       GAAAACAAGGGATCGGTTTAGATTTTTCCCCAAAATTTAAATTAAATTTCAGATGACATC
  6961 ---------+---------+---------+---------+---------+---------+  7020
                                                             M  T  S
```

FIGURE 3(g)

```
      CCGCCTGCTCAAAAAGTTCTACTTTTGGCCGGAAGCACGAAACTCTGCCGTCTAAAATTC
7021  ---------+---------+---------+---------+---------+---------+  7080
       R  L  L  K  K  F  Y  F  W  P  E  A  R  N  S  A  V  *
                490                           500

      ACTCGTGATTCATTGCCCAATTGATAATTGTCTGTATCTTCTCCCCCAGTTCTCTTTCGC
7081  ---------+---------+---------+---------+---------+---------+  7140
      CCAATTAGTTTAAAACCATGTGTATATTGTTATCCTATACTCATTTCACTTTATCATTCT
7141  ---------+---------+---------+---------+---------+---------+  7200
      ATCATTTCTCTTCCCATTTTCACACATTTCCATTTCTCTACGATAATCTAAAATTATGAC
7201  ---------+---------+---------+---------+---------+---------+  7260
      GTTTGTGTCTCGAACGCATAATAATTTTAATAACTCGTTTTGAATTTGATTAGTTGTTGT
7261  ---------+---------+---------+---------+---------+---------+  7320
      GCCCAGTATATATGTATGTACTATGCTTCTATCAACAAAATAGTTTCATAGATCATCACC
7321  ---------+---------+---------+---------+---------+---------+  7380
      CCAACCCCACCAACCTACCGTACCATATTCATTTTTGCCGGGAATCAATTTCGATTAATT
7381  ---------+---------+---------+---------+---------+---------+  7440
      TTAACCTATTTTTTCGCCACAAAAAATCTAATATTTGAATTAACGAATAGCATTCCCATC
7441  ---------+---------+---------+---------+---------+---------+  7500
      TCTCCCGTGCCGGAATGCCTCCCGGCCTTTTAAAGTTCGGAACATTTGGCAATTATGTAT
7501  ---------+---------+---------+---------+---------+---------+  7560
      AAATTTGTAGGTCCCCCCCATCATTTCCCGCCCATCATCTCAAATTGCATTCTTTTTTCG
7561  ---------+---------+---------+---------+---------+---------+  7620
      CCGTGATATCCCGATTCTGGTCAGCAAAGATCT
7621  ---------+---------+---------+---  7653
```

FIGURE 3(h)

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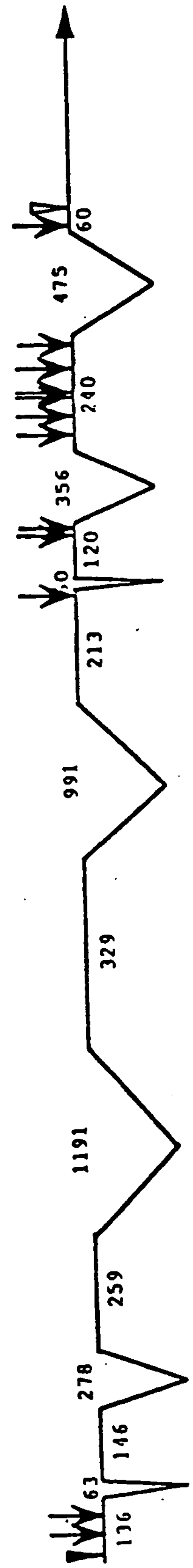

FIGURE 4(a)

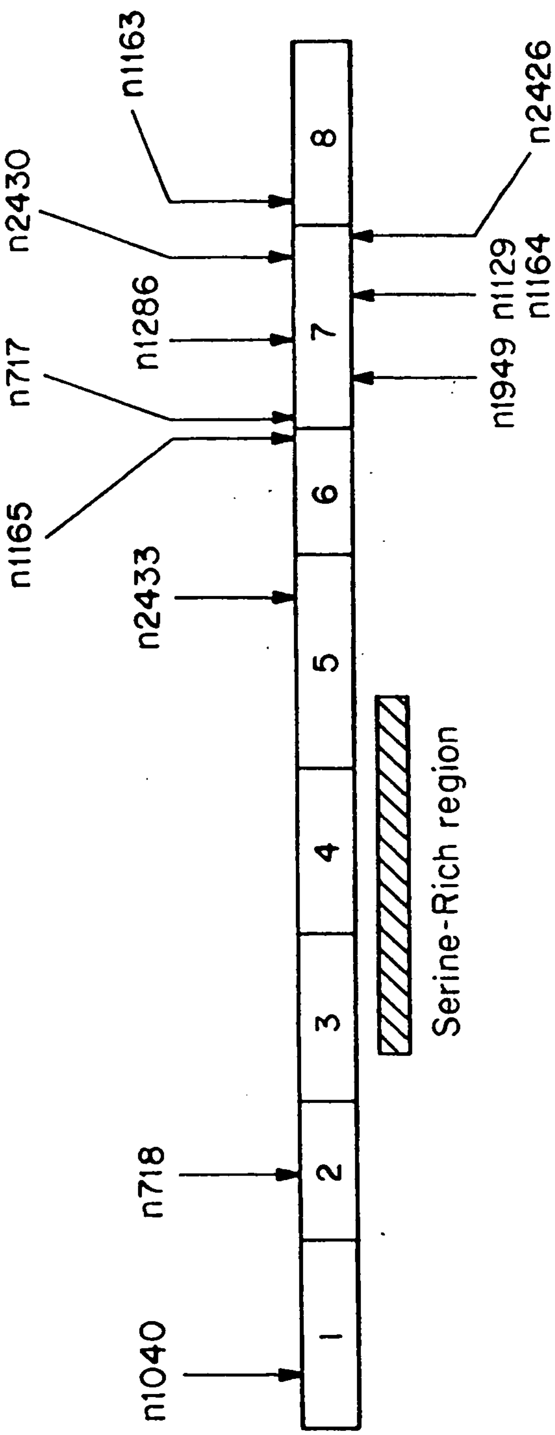
n1163
n2430
n1286
n717
n1165
n2433
n718
n1040
n2426
n1129
n1164
n1949
8
7
6
5
4
3
2
1
Serine-Rich region

FIG. 4B

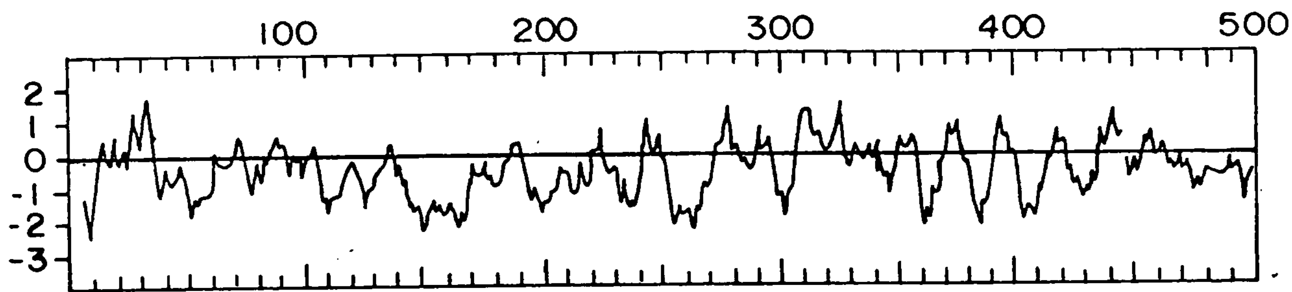

FIG. 5

Alignment of Ced-3 and Human Interleukin-1β Convertase

```
ICE     1     MADKVLKEKRKLFIRSM....GEGTINGLLDELLQTRVLNKEEMEKVKRE
                 .: .::|.|: |.:    :. .::::|: |:...|||.:: :.:. .
Ced-3   1     ...MMRQDRRSLLERNIMMFSSHLKVDEILEVLIAKQVLNSDNGDMIN.S
                                           ↓
                                           F
BGAFQ            ===============================================
PBA              ===============================================


        47    NATVMDKTRALIDSVIPKGAQACQ.ICITYICEEDSYLAGTLGLSADQTS
               :||.:| |.::..| ..|. | : :: .. :.:.. ||:.|:  |  ..
        47    CGTVREKRREIVKAVQRPGDVAFDAFYDALRSTGHEGLAEVLEPLARSVD
                                ↓
                                R
BFAFQ         ==================================================
PBA           ==================================================


                                               autocleavage site
        96    GNYLNMQ...........................DSQGVLSSF.......
              :| ::::                           || : :|||
        97    SNAVEFECPMSPASHRRSRALSPAGYTSPTRVHRDSVSSVSSFTSYQDIY
                                      serine-rich region
BGAFQ         ==================================================
PBA           ==================================================


        112   ......................PAPQAVQDNPAMPTSSGSEGNVKLCSLE
                                    |:..|....|. :.|| .:..   :| .
        147   SRARSRSRSRALHSSDRHNYSSPPVNAFPSQPSSANSSFTGCSSLGYSSS

BGAFQ         ==================================================
PBA           ===


        140   EAQRIWKQKSAEIYPIMDK.......................SSRTRLAL
               ...:.|..::. | : :.                       ||.  :.|
        197   RNRSFSKASGPTQYIFHEEDMNFVDAPTISRVFDEKTMYRNFSSPRGMCL

BGAFQ         ==================================================
```

FIGURE 6(a)

```
ICE     167  IICNEEFDSIPRRTGAEVDITGMTMLLQNLGYSVDVKKNLTASDMTTELE
             || ||.|: :| |.|...| ..:| |:. :||.| .|.|||:.:|  .:
Ced-3   247  IINNEHFEQMPTRNGTKADKDNLTNLFRCMGYTVICKDNLTGRGMLLTIR

BGAFQ        ==================================================


        217  AFAHRPEHKTSDSTFLVFMSHGIREGICGKKHSEQVPDI.LQLNAIFNML
             .||.:..|  :||.:||::|||  :.|.|      |.|| :  :.|:::|
        297  DFAKHESH..GDSAILVILSHGEENVIIG......VDDIPISTHEIYDLL

BGAFQ        ==================================================


                              active site autocleavage site
        266  NTKNCPSLKDKPKVIIIQACRGDSPGVVW.FKDSVGVSGNLSLPTTEEFE
             |. |.|.| :|||::::|||||:..: .: . |||:. ..:   .. :: :
        339  NAANAPRLANKPKIVFVQACRGERRDNGFPVLDSVDGVPAFLRRGWDNRD
                                  ↓
                                  S
BGAFQ        =================================


        315  DDAI..............KKAHIEKDFIAFCSSTPDNVSWRHPTMGSVFI
             :. :              :| . : |::  :..|:: ||||:...|| ||
        389  GPLFNFLGCVRPQVQQVWRKKPSQADILIRYATTAQYVSWRNSARGSWFI
                                    ↓               ↓
                                   stop            stop


        351  GRLIEHMQEYACSCDVEEIF....RKVRFSFEQPDGRAQMPTT.ERVT.L
             . :.| : ..| . || |::    :||  :|: .:|.. :.   | .: |
        439  QAVCEVFSTHAKDMDVVELLTEVNKKVACGFQTSQGSNILKQMPEMTSRL
                       ↓                ↓                ↓  ↓
                       V                V                K  F


        395  TRCFYLFPGH*.... 404
              : ||::|:
        489  LKKFYFWPEARNSAV 503
```

FIGURE 6(b)

```
251 EHFEQMPTRNGTKADKDNLTNLFRCMGYTVICKDNLTGRGMLLTIRDFAK 300
                                             :||:. : .
  1 .........................................MLTVQVYRT 9

301 HESHGDSAILVILSHGEENVIIGVDDIPISTHEIYDLLNAANAPRLANKP 350
     :. :.| :|        :|:::    |::| .::.||          .|
 10 SQKCSSSKHVV.......EVLLD....PLGT.SFCSLL..........PP 37

351 KIVFVQACRGERRDNGFPVLDSVDGVPAFLRRGWDNRDGPLFNFLGCVRP 400
    .::: :. || .::|    :|.:.          ::.|:.  :::
 38 PLLLYETDRGVDQQDGKNHTQSPGC.........EESDAGKEELM..... 73

401 QVQCVWRKKPSQADILIRYATTAQYVSWRNSARGSWFIQAVCEVFSTHAK 450
         ..: |...|::. ||.  .  .. ||. ||||:|:|:..:|||.:|
 74 .....KMRLPTRSDMICGYACLKGNAAMRNTKRGSWYIEALTQVFSERAC 118

451 DMDVVELLTEVNK..KVACGFQTSQGSNILKQMPEMTSRLLKKFYFWPEA 498
    ||.|.::|..||   |    |:..: : :  |:|.| .| | ..:|::|:
119 DMHVADMLVKVNALIKEREGYAPGTEFHRCKEMSEYCSTLCQQLYLFPGY 168

499 RNSAV 503

169 PPT*. 172
```

FIGURE 7

Alignment of N-terminal regions of ced-3/ICE- related proteins

```
C. briggsae ced-3   MMRQDRWSLLERNILEFSSKLQADLILDVLIAKQVLNSDNGDVINSCRTERDNEKEIVKAVQRRGDEAFDAFYDALRDTGHNDLADVLMPLSR---PNPV
ced-3 protein       MMRQDRRSLLERNIMMFSSHLKVDEILEVLIAKQVLNSDNGDMINSCGTVREKRREIVKAVQRPGDVAFDAFYDALRSTGHEGLAEVLEPLARSVDSNAV 100
C. vulgaris ced-3   ----------------------------------------------------------------------------------------------------
Mouse ICE.gw        M-------------------ADKIL---------------------RAKRKQFINSV---SIGTINGLLDELLEK------RVLNQEEM----DKI
Human ICE.GW        M-------------------ADKVL---------------------KEKRKLFIRSM---GEGTINGLLDELLQT------RVLNKEEM----EKV

Consensus           M-------------------AD.IL---------------------R.KRK.....V---G.......D.L..T------.VL.........-..V

C. briggsae ced-3   PMECPMSPSSHRRSRALSPPGYASPTRVHRDSISSVSSFTSTYQDVYSRARSSSRSSRPLQSSDRHNYMSAA-TSFPSQPSSANSSFTGCASLGYSSSRN
ced-3 protein       EFECPMSPASHRRSRALSPAGYTSPTRVHRDSVSSVSSFTSYQD-IYSRARSRSRS-RALHSSDRHNYSSPPVNAFPSQPSSANSSFTGCSSLGYSSSRN 198
C. vulgaris ced-3   ----------------------------------------------STSRSSRPLHTSDRHNYVSPS-NSFQSQPASANSSFTGSSSLGYSSSRT
Mouse ICE.gw        KLA---NITAMDKARDLCDHVSKKGPQASQIFITYICNEDCYL--------AGILELQSAPSAE--TFVAT------EDSKGGHPSSSETKEEQNKED-G
Human ICE.GW        KRE---NATVMDKTRALIDSVIPKGAQACQICITYICEEDSYL--------AGTLGLSADQTSG--NYLNM------QDSQGVLSSFPAPQAVQDNPAMP

Consensus           ..E--.........RAL...............I.......SY.--------S.SRS.R.L.SSDRHNY.S..-..F.SQP.SANSSFTG..SLGYSSSR.

C. briggsae ced-3   RSFSKTSAQSQYIFHEEDMNYVDAPTIHRVFDEKTMYRNFSSPRGLCLIINNEHFEQMPTRNGTKADKDNLTNIFRCMGYTVICKDNLTGREMLSTIRSF
ced-3 protein       RSFSKASGPTQYIFHEEDMNFVDAPTISRVFDEKTMYRNFSSPRGMCLIINNEHFEQMPTRNGTKADKDNLTNLFRCMGYTVICKDNLTGRGMLLTIRDF 298
C. vulgaris ced-3   RSYSKASAHSQYIFHEEDMNYVDAPTIHRVFDEKTMYRNFSTPRGLCLIINNEHFEQMPTRNGTKPDKDNISNLFRCMGYIVHCKDNLTGRMML-TIRDF
Mouse ICE.gw        TFPGLTGTLKFCPLEKAQKLWKENPS--EIY--PIMNTT-TRTRLALIINNTEFDHLSFRNGAQVDLREMKLLLEDLGYTVKVKENLTALEMVKEVKEF
Human ICE.GW        TSSGSEGNVKLCSLEEAQRIWKQKSA--EIY--PIMDKS-SRTRLALIINNEHFDSIPRRTGAEVDITGMTMLLQNLGYSVDVKKNLTASDMTTELEAF

Consensus           RS.SK.S...QYIFHEEDMN.VDAPTI.RVFDEKTMYRNFS.PRGLCLIINNEHFEQMPTRNGTK.DKDN.TNLFRCMGYTVICKDNLTGR.ML.TIR.F

C. briggsae ced-3   GRNDMH--GDSAILVILSHGEENVIIG---VDDVS--VNVHEIYDLLNAANAPRLANKPKLVFVQACRG
ced-3 protein       AKHESH--GDSAILVILSHGEENVIIG---VDDIP--ISTHEIYDLLNAANAPRLANKPKIVFVQACRG 360
C. vulgaris ced-3   AKNETH--GDSAILVILSHGEENVIIG---VDDVS--VNVHEIYDLLNAANAPRLANKPKLVFVQACRG
Mouse ICE.gw        AACPEHKTSDSTFLVFMSHGIQEGICGTTYSNEVSDILKVDTIFQMMNTLKCPSLKDKPKVIIIQACRG
Human ICE.GW        AHRPEHKTSDSTFLVFMSHGIREGICGKKHSEQVPDILQLNAIFNMLNTKNCPSLKDKPKVIIIQACRG

Consensus           A....H--GDSAILVILSHGEENVIIG---VDDVS--..VHEIYDLLNAANAPRLANKPK.VFVQACRG
```

FIGURE 8(a)

Alignment of the C-terminal regions of ced-3/ICE/NEDD-2 - related proteins

```
ICE C-terminus           DSPGVVW--- ---------- --FKDSVG-- ---------- ---------V
Mouse ICE C-ter          EKQGVVL--- ---------- --LKDSVR-- ---------- ---------D
C.briggsae C-ter         ERRDNGFP-- ---------- --VLDSVDG- -----VPSLI ----RRGWDN
ced-3 Cterminus          ERRDNGFP-- ---------- --VLDSVDG- -----VPAFL ----RRGWDN 386
C. vulgaris C-terminus   ERRDVGFP-- ---------- --VLDSVDG- -----VPALI ----RRGWDK
nedd-2 protein.gw        MLTVQVYRTS QKCSSSKHVV EVLLDPLGTS FCSLLPPPLL LYETDRGVDQ

Consensus                E.......-- ---------- --.LDSV..- -----.P... ----.RG.D.

ICE C-terminus           SGNL---SLP TTEEFE---D DAIKKA-HIE KDFIAFCSST PDNVSWRHPT
Mouse ICE C-ter          SEE----DFL TDAIFE---D DGIKKA-HIE KDFIAFCSST PDNVSWRHPV
C.briggsae C-ter         RDG-PLFNFL GCVRPQV--Q QVWRKK-PSQ ADMLIAYATT AQYVSWRNSA
ced-3 Cterminus          RDG-PLFNFL GCVRPQV--Q QVWRKK-PSQ ADILIRYATT AQYVSWRNSA 432
C. vulgaris C-terminus   GDG-P--NFL GCVRPQA--Q QVWRKK-PSQ ADILIAYATT AQYVSWRNSA
nedd-2 protein.gw        QDGKNHTQSP GCEESDAGKE ELMKMRLPTR SDMICGYACL KGNAAMRNTK

Consensus                .DG-....FL GC.....--. ....K.-P.. .D....YA.T ...VSWRN..

ICE C-terminus           MGSVFIGRLI EHMQEYACSC DVEEIFRKV- -RF----SFE QPDGRAQMPT
Mouse ICE C-ter          RGSLFIESLI KHMKEYAWSC DLEDIFRKV- -RF----SFE QPEFRLQMPT
C.briggsae C-ter         RGSWFIQAVC EVFSLHAKDM DVVELLTEVN KKVA--CGFQ TSQGSNILKQ
ced-3 Cterminus          RGSWFIQAVC EVFSTHAKDM DVVELLTEVN KKVA--CGFQ TSQGSNILKQ 480
C. vulgaris C-terminus   RGSWFIQAVC EVFSLHAKDM DVVELLTEVN KKVA--CGFQ TSQGANILKQ
nedd-2 protein.gw        RGSWYIEALT QVFSERACDM HVADMLVKVN ALIKEREGYA PGTEFHRCKE

Consensus                RGSWFI.A.. EVFS..A.DM DV.E.L..VN ....--.GF. ...G....K.

ICE C-terminus           T_ERVT-LTR CFYLFPGH-- -----
Mouse ICE C-ter          A_DRVT-LTK RFYLFPGH-- -----
C.briggsae C-ter         MPELTSRLLK KFYFWPEDRG RNSAV
ced-3 Cterminus          MPEMTSRLLK KFYFWPEAR- -NSAV 503
C. vulgaris C-terminus   MPELTSRLLK KFYFWPEDRN RSSAV
nedd-2 protein.gw        MSEYCSTLCQ QLYLFPG--- -YPPT

Consensus                M.E.TS.L.K .FY..P...- -....
```

FIGURE 8(b)

```
Lines

1    01 MMRQDRRSLLERNIMMFSSHLKVDEILEVLIAKQVLNSDNGDMINSCGTV  50
2       ......W_......LE...K.QA.L..D..............V....R.E
3                                TVSISLJ..R.........M.....

1    51 REKRREIVKAVQRPGDVAFDAFYDALRSTGHEGLAEVLEPLARSVDSNAV 100
2       .DNEK........R..E..........D...ND..D..M..S.P    .P.
3

1   101 EFECPMSPASHRRSRALSPAGYTSPTRVHPDSVSSVSSFTS_YQDIYSRA 149
2       PM......S..........P .A.........I........T...V....
3                                                        S

1   150 RSRSR_SRALHSSDRHNYSSPPVNAFPSQPSSANSSFTGCSSLGYSSSRN 198
2       ..S..S..P.Q.......M.AA_TS.....  .........A.........
3       T...__..P..T......V..S_.S.Q...A........S.........T

1   199 RSFSKASGPTQYIFHEEDMNFVDAPTISRVFDEKTMYRNFSSPRGMCLI 247
2       .....T.ACS..........Y......H.................L...
3       ..Y....AHS..........Y......H.............T...L...

1   248 INNEHFECMPTRNGTKADKDNLTNLFRCMGYTVICKDNLTGRGMLLTIRD 297
2       ...........................I.................E..S...S
3       ...............P....IS.........I.H........  .M.....

1   298 FAKHESHGDSAILVILSHGEENVIIGVDDIPISTHEIYDLLNAANAPRLA 347
2       .GRNDM..............  ........VSVNV...............
3       ...N.T.........  ..............VSVNV....X...........

1   348 NKPKIVFVCACRGERPDNGFPVLDSVDGVPAFLRRGWDNRDGPLFNFLGC 397
2       ....L........................SLI..................
3       ....L............V.............LI.....KG...   .....

1   398 VRPQVQQVWRKKPSQADILIRYATTAQYVSWRNSARGSWFIQAVCEVFST 447
2       ..................M..A...........................L
3       ....A...............A............................L

1   448 HAKDMDVVELLTEVNKKVACGFQTSCGSNILKQMPEMTSRLLKKFYFWPE 497
2       ..................................L..............
3       ..........................A.......  L............

1   498 __ARN__SAV 503
2       DRG..__...
3       __D..RS...
```

FIGURE 9

Interleukin-1β convertase cDNA seqeunce

```
   1 AAAAGGAGAG AAAAGCCATG GCCGACAAGG TCCTGAAGGA GAAGAGAAAG
  51 CTGTTTATCC GTTCCATGGG TGAAGGTACA ATAAATGGCT TACTGGATGA
 101 ATTATTACAG ACAAGGGTGC TGAACAAGGA AGAGATGGAG AAAGTAAAAC
 151 GTGAAAATGC TACAGTTATG GATAAGACCC GAGCTTTGAT TGACTCCGTT
 201 ATTCCGAAAG GGGCACAGGC ATGCCAAATT TGCATCACAT ACATTTGTGA
 251 AGAAGACAGT TACCTGGCAG GGACGCTGGG ACTCTCAGCA GATCAAACAT
 301 CTGGAAATTA CCTTAATATG CAAGACTCTC AAGGAGTACT TTCTTCCTTT
 351 CCAGCTCCTC AGGCAGTGCA GGACAACCCA GCTATGCCCA CATCCTCAGG
 401 CTCAGAAGGG AATGTCAAGC TTTGCTCCCT AGAAGAAGCT CAAAGGATAT
 451 GGAAACAAAA GTCGGCAGAG ATTTATCCAA TAATGGACAA GTCAAGCCGC
 501 ACACGTCTTG CTCTCATTAT CTGCAATGAA GAATTTGACA GTATTCCTAG
 551 AAGAACTGGA GCTGAGGTTG ACATCACAGG CATGACAATG CTGCTACAAA
 601 ATCTGGGGTA CAGCGTAGAT GTGAAAAAAA ATCTCACTGC TTCGGACATG
 651 ACTACAGAGC TGGAGGCATT TGCACACCGC CCAGAGCACA AGACCTCTGA
 701 CAGCACGTTC CTGGTGTTCA TGTCTCATGG TATTCGGGAA GGCATTTGTG
 751 GGAAGAAACA CTCTGAGCAA GTCCCAGATA TACTACAACT CAATGCAATC
 801 TTTAACATGT TGAATACCAA GAACTGCCCA AGTTTGAAGG ACAAACCGAA
 851 GGTGATCATC ATCCAGGCCT GCCGTGGTGA CAGCCCTGGT GTGGTGTGGT
 901 TTAAAGATTC AGTAGGAGTT TCTGGAAACC TATCTTTACC AACTACAGAA
 951 GAGTTTGAGG ATGATGCTAT TAAGAAAGCC CACATAGAGA AGGATTTTAT
1001 CGCTTTCTGC TCTTCCACAC CAGATAATGT TTCTTGGAGA CATCCCACAA
1051 TGGGCTCTGT TTTTATTGGA AGACTCATTG AACATATGCA AGAATATGCC
1101 TGTTCCTGTG ATGTGGAGGA AATTTTCCGC AAGGTTCGAT TTTCATTTGA
1151 GCAGCCAGAT GGTAGAGCGC AGATGCCCAC CACTGAAAGA GTGACTTTGA
1201 CAAGATGTTT CTACCTCTTC CCAGGACATT AAAATAAGGA AACTGTATGA
1251 ATGTCTGCGG GCAGGAAGTG AAGAGATCGT TCTGTAAAAG GTTTTTGGAA
1301 TTATGTCTGC TGAATAATAA ACTTTTTTTG AAATAATAAA TCTGGTAGAA
1351 AAATGAAAAA AAAAAAAAAA AAA
```

FIGURE 10

Constructs that Prevent Programmed Cell Death in C. elegans

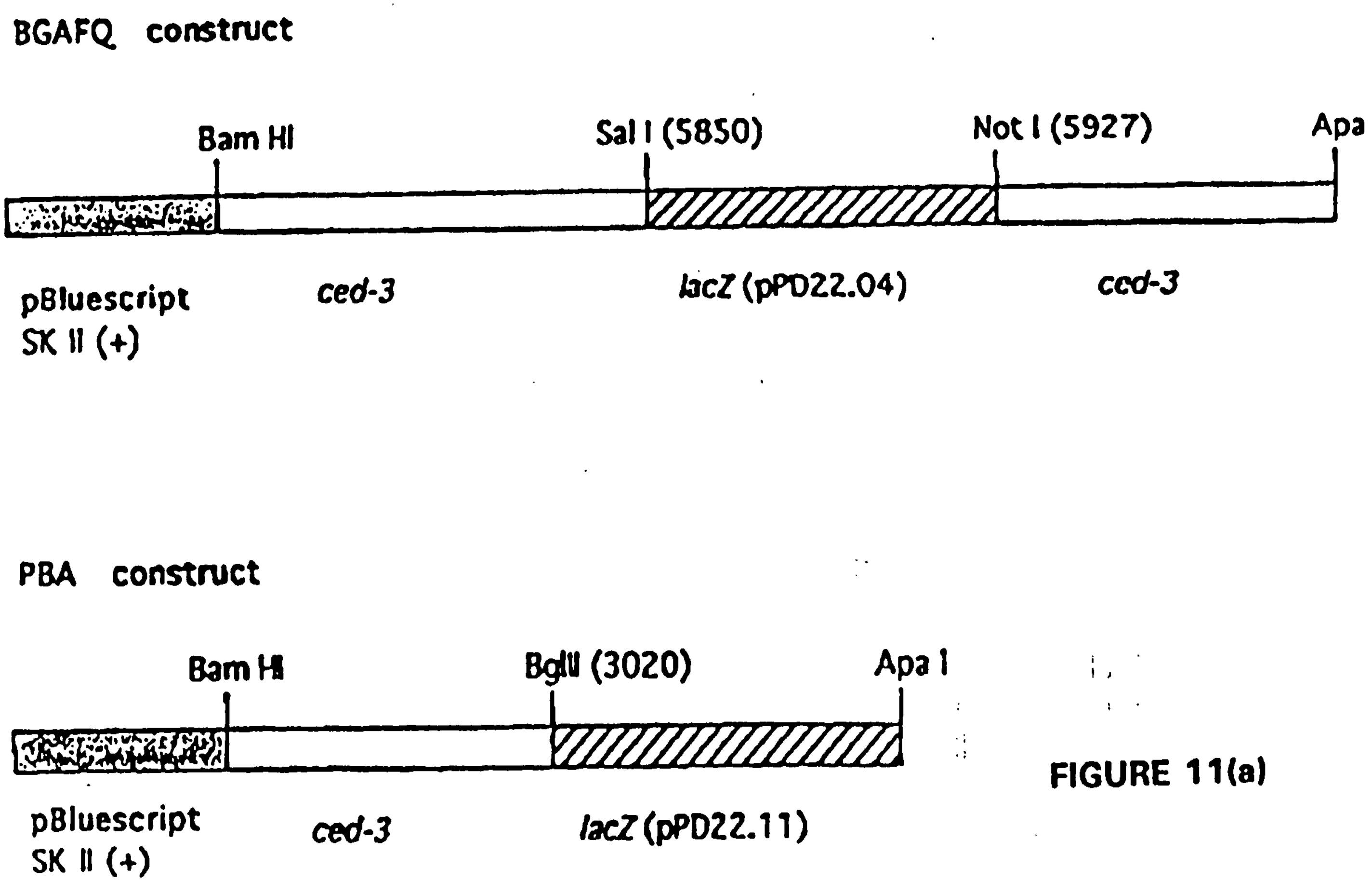

FIGURE 11(a)

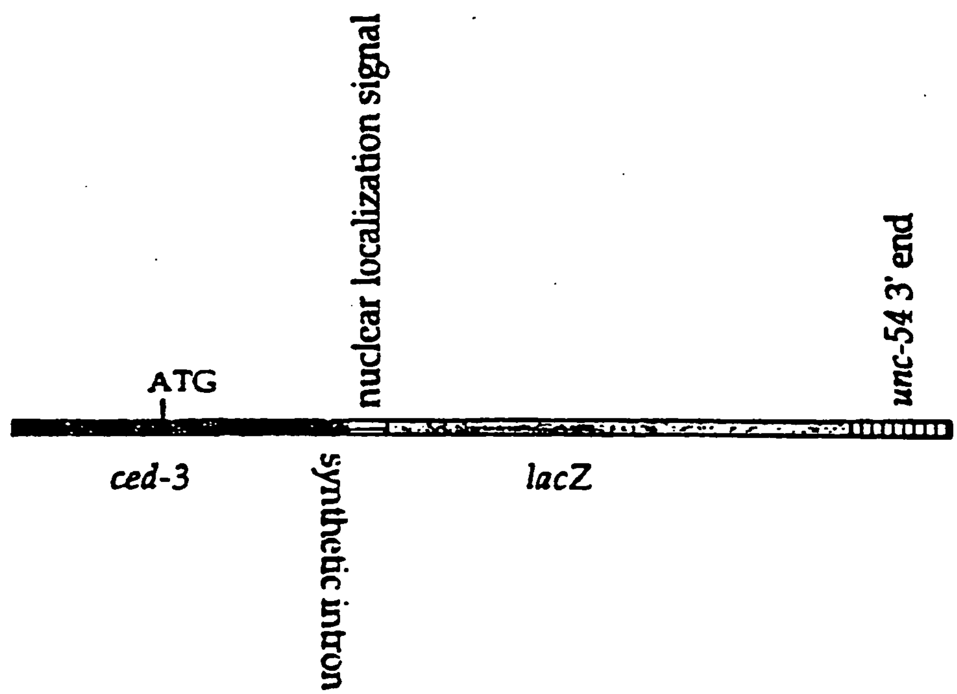
nuclear localization signal
unc-54 3' end
ATG
ced-3
synthetic intron
lacZ

FIGURE 11(b)

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 93/05705

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 C12N15/57; C12N9/64; C12N15/39; A61K31/70
A61K37/02; C12Q1/68; G01N33/577; G01N33/68

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	C12N ; A61K ; C12Q ; G01N

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No.[13]
Y	DISSERTATION ABSTRACT INTERNATIONAL B vol. 50, no. 10, April 1990, page 4431-B J. YUAN 'Genetic and molecular studies of ced-3 and ced-4: Two genes that control programmed cell death' see page 4431-B, left column, paragraph 2 abstract ---	1-13, 16-21, 28-45, 101-122
Y	WO,A,9 207 071 (THE BOARD OF TRUSTEES OF THE UNIVERSITY OF ILLINOIS) 30 April 1992 see page 6, line 1 - page 7, line 30; claims 1-27 ---	1-45, 101-122

-/--

° Special categories of cited documents : [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

1

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
05 OCTOBER 1993	15 -10- 1993
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	HORNIG H.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
X	WO,A,9 115 577 (IMMUNEX CORPORATION) 17 October 1991	54-56
Y	see page 3, line 25 - page 6, line 10; claims 1-30	14,15, 22-27, 101-122

X	NATURE vol. 356, 30 April 1992, MACMILLAN JOURNALS LTD., LONDON,UK; pages 768 - 774 N.A. THORNBERRY ET AL. 'A novel heterodimeric cystein protease is required for interleukin-1-beta processing in monocytes' cited in the application	54-57
Y	see page 770, right column, paragraph 2	14,15, 22-27, 101-122
	see page 773, right column, paragraph 2	

X	CELL vol. 69, no. 4, 15 May 1992, CELL PRESS, CAMBRIDGE, MA,US; pages 597 - 604 C.A. RAY ET AL. 'Viral inhibition of inflammation: Cowpox virus encodes an inhibitor of the interleukin-1-beta converting enzyme' cited in the application see page 599, right column, paragraph 2 - page 600, left column, paragraph 1	54,58

A	ANNUAL REVIEW OF CELL BIOLOGY vol. 7, 1991, ANNUAL REVIEW INC.,PALO ALTO,CA,US; pages 663 - 698 R.E. ELLIS ET AL. 'Mechanisms and functions of cell death' cited in the application the whole document	-
	---	
P,X	BIOCHEM. BIOPHYS. RES. COMMUN. vol. 185, no. 3, 30 June 1992, ACADEMIC PRESS, N.Y., US; pages 1155 - 1161 S. KUMAR ET AL. 'Identification of a set of genes with developmentally down-regulated expression in the mouse brain' cited in the application see page 1157, paragraph 4 - page 1161, paragraph 1; table 1	86,87

	-/--	

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
P,X	DATABASE WPI Week 9318, Derwent Publications Ltd., London, GB; AN 93-144400 & CA,A,2 076 159 (MERCK & CO. INC.) 17 February 1993 see abstract ---	128
P,X	EP,A,0 533 350 (MERCK & CO. INC.) 24 March 1993 abstract ---	14,[illegible], [illegible]24, [illegible],42, 60,128
P,Y	NEUROBIOLOGY OF AGING vol. 13, 1992, PERGAMON PRESS, NEW YORK, US; page S47 S. LEDOUX AND J. YUAN 'Isolation of nematode homologs of the C. elegans cell death gene ced-3' Third international conference on Alzheimer's disease and related disorders, Abano terme, Italy, July 12-17, 1992; Abstract no. 183; abstract -----	10[illegible]

INTERNATIONAL SEARCH REPORT	International application No. PCT/US93/05705

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark . Although claims 65,66,71,72 (as far as they concern in vivo treatment of human or animals) and claims 51,63,77,78 are directed to a method of treatment of the human/animal body the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.

US 9305705
SA 76331

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information. 05/10/9

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9207071	30-04-92	US-A-	5217889	08-06-93
		AU-A-	9025091	20-05-92
		CA-A-	2094144	20-04-92
		EP-A-	0555370	18-08-93
WO-A-9115577	17-10-91	AU-A-	7775991	30-10-91
EP-A-0533350	24-03-93	None		

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82